THE IMPACT OF INOCULATION WITH *PROPIONIBACTERIUM* ON RUMINAL ACIDOSIS IN BEEF CATTLE

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FORMAT OF THESIS

This thesis is prepared as outlined by the Oklahoma State University graduate college style manual. This thesis is presented in the Journal of Animal Science style format. The use of this format allows for independent chapters to be suitable for submission to scientific journals. Each paper is complete in itself with an abstract, introduction, materials and methods, results and discussion, implications, and literature cited section.

CHAPTER I.

INTRODUCTION

Ruminal acidosis has been a common production problem facing the cattle feeding industry. Feedlot cattle can exhibit signs of acidosis during the adaptation to a concentrate diet or during excessive intake in the feeding period. This digestive disorder can be acute or subacute with either overt illness or retarded performance, respectively. The etiology of ruminal acidosis has been described in excellent reviews by Elam (1976), Slyter (1976), Huntington (1988), and Owens et al. (1996).

Research has been conducted in a number of areas such as grain processing, feeding management, dietary cation-anion balance, narrow-spectrum antibiotics, and lactate-utilizing microbes to reduce the incidence of acute and subacute acidosis. The goal of this research was to investigate the value of propionibacteria as a direct fed microbe to aid in preventing acidosis that accompanies adaptation to a high concentrate diet. Chapter two reviews the literature on ruminal acidosis and provides an overview of management procedures used to control acidosis. Chapter three contains the results of a trial determining establishment of propionibacteria strains inoculated in cows. Chapter four presents the results of three trials conducted to determine the utility of a propionibacteria co-culture inocula during rapid adaptation periods. Chapter five contains the results of an acute acidosis trial that to explored the etiology of acute acidosis. Chapter six presents the results of a study using propionibacteria strain P-63 as an inoculant to prevent acidotic incidences during a rapid adaptation to a high concentrate diet that contained a large percentage of wheat. Chapter seven contains the results of a feedlot trial in which

propionibacteria strain P-63 was tested alone or in combination with a lactobacillis strain. Finally, chapter eight summarizes all of this research.

CHAPTER II.

REVIEW OF LITERATURE

OVERVIEW OF RUMINAL ACIDOSIS

Acidosis is a digestive disturbance of the rumen. Among the names used to describe this digestive disorder are overloaded rumen, engorgement toxemia and acute indigestion. In 1965, the term 'D-lactic acidosis' was devised by Dunlop and Hammond. In more recent years, acidosis has been divided based on severity into conditions called acute acidosis and subacute (chronic) acidosis. Although these two terms are used clinically to describe the time course of the disorder, etiology and impact of acute and chronic acidosis differ just as toxicology of chronic and acute poisoning will differ.

Acidosis is most prominent in cattle that accidently gain access to a large quantity of a concentrate. This is considered acute acidosis. Acute acidosis also occurs in the feedlot industry when the cattle is being switched from roughage to concentrate. In feedlots, acute acidosis becomes apparent when animals die while with chronic acidosis, cattle may merely exhibit a reduced feed intake. Animals with acute acidosis exhibit overt illness; with subacute acidosis, animals may not appear ill but simply have decreased feed intake and performance (Slyter,1976). Although much of the information on acidosis has been obtained from the acute condition that occurs when rumen-fistulated animals engorge (voluntarily or manually) grains or other carbohydrates, voluntary grain engorgement occurs frequently enough in feedlots to have economic importance.

Clinically, acidosis is the result of a combination of two separate but interrelated events (Johnson, 1991). The first is excessive ingestion of rapidly fermented carbohydrates. The second is conversion of these carbohydrates to

lactic acid or volatile fatty acids (VFA) that decrease ruminal pH. Other symptoms that may be casually or causally related include an elevated ruminal glucose concentration, decreased ruminal motility, increased ruminal osmolality, and decreased salivation. An increased ruminal osmolality causes the animal to become dehydrated and decrease salivation;this decreases ruminal input of salivary buffers, exacerbating the accumulation of ruminal acids. Eliminating either excess carbohydrate intake or ruminal acidity will avoid ruminal acidosis.

Ruminal bacteria rapidly ferment starch from cereal grains; acidic fermentation end-products are responsible for acidosis. When supply of starch increases abruptly, both VFA and lactic acid will increase in concentration. Either lactate or VFA can decrease ruminal pH. With subacute acidosis, several workers (Harmon et al., 1985; Burrin and Britton, 1986; Goad, 1990) have proposed that VFA play the most prominent role, but with acute acidosis, lactate is usually most prominent. Although normally present at low concentrations in the rumen, lactic acid concentrations during acidosis can reach 100 mM (Kezar and Church, 1979). Other toxic factors including histamine, tyramine and tryptamine, ethanol and bacterial endotoxins also can appear and may play roles in acidosis (Slyter, 1976).

Etiology of Ruminal Acidosis

Ruminal contents typically range in pH from 5.5 to 7.5. With concentrate (feedlot) diets and rapid starch fermentation, ruminal pH will be low. With higher roughage diets, ruminal pH is higher, often near 7.0. Cellulose fermentation is reduced or absent when pH falls below 6.0. The ruminal concentration of VFA generally is greater with fermentation of starch than of cellulose because 1) rate and extent of fermentation generally is greater, 2) ruminal volume is less, and 3) ruminal contents are less dilute because salivary input is lower.

VFA and Lactate. Ingestion of rapidly and extensively fermented cereal grains increases the ruminal prevalence of amylolytic bacteria. The predominantly gram-negative cellulolytic ruminal flora is displaced by more gram-positive bacteria after animals ingest large amounts of rapidly fermented

carbohydrate. Certain strains (*Streptococcus bovis* and *Lactobacillus* species) of ruminal bacteria rapidly ferment available carbohydrate to VFA and(or) lactic acid. Normally, lactic acid is produced slowly enough that its metabolism keeps pace with its production (Johnson, 1991). Dunlop (1961) noted that lactate disappearance in the rumen is pH dependent. When ruminal pH is normal (6.6 - 7.0), lactate is removed twice as fast as at a low pH (3.5 - 6.4), due partly to depression of lactate dehydrogenase activity at a low pH as described by Russell and Hino (1985) and DeVries et al. (1970). These studies suggest that lactate dehydrogenase activity was maximum at pH 5.5 and less when pH reached 4.7. Starch fermentation also increases production of VFA; these are acids but, because of their higher pK, they depress ruminal pH less. Ruminal pH values with lactic acidosis can drop as low as 3.9 to 4.5 (Dunlop, 1972). The shift in the bacterial population and decreased ruminal pH severely inhibits activity and prevalence of cellulolytic microbes of the rumen. Acidosis also can depress or destroy the ruminal protozoa (Slyter,1976); Purser and Moir (1959) found that protozoal activity and reproduction were inhibited when pH fell to 5.5.

Lactic acid is the dominant force in acute acidosis, being ten times stronger than VFA. The pK of lactic acid is 3.7 (Dunlop, 1972) versus 4. 7 to 4.8 for VFA. However, with subacute acidosis, lactate may not play a major role as compared to VFA because lactate seldom is detected in cases of subacute acidosis. Thereby, the range in pH of the rumen during acute and subacute acidosis is different. During subacute acidosis, ruminal pH normally ranges from 5.0 and 5.6 (Horn et al., 1979; Britton, 1985; Burrin and Britton, 1986) whereas during acute acidosis, ruminal pH often decreases to 3.9 to 4.5 (Hungate, 1966; Dunlop, 1972). Acute acidosis has been defined as the condition of a ruminal pH below 5.0 (Owens et al., 1996)

Lactate is absorbed only slowly across a healthy rumen wall when pH is near neutrality. However, when ruminal lactic acid concentration increases and ruminal pH decreases to 4.0, rate of lactic acid absorption is increased. Some of the absorbed lactate is metabolized by the liver. Although L-isomer of lactate is

easily metabolized, the D-isomer of lactate is metabolized more slowly. Due to its accumulation of lactate in blood, Dunlop and Hammond (1965) coined the term 'D-lactic acidosis' for the acute ruminal acidosis. Huntington et al. (1981) found that net portal D-lactate absorption increased 1.9 times as concentrate intake increased even though mean arterial plasma D-lactate concentration decreased slightly.

Blood Parameters. Lactate, when produced rapidly, quickly overrides the buffering capacity in the rumen. Besides increasing absorption of lactate, a low ruminal pH causes more dissociation of VFA which leads to more rapid absorption of VFA. Combined, lactate and VFA can overwhelm the bicarbonate buffering of blood resulting in metabolic acidosis. When lactic acid enters the blood and dissociates to form an anion and a hydrogen ion, the anion combines with sodium and H^+ combines with bicarbonate to form carbonic acid which dissociates into CO_2 and H_2O. Overall, this decreases bicarbonate concentration and increases CO_2. With metabolic acidosis, blood bicarbonate and base excess both decrease. In turn, the animal hyperventilates to rid the body of excess CO_2. Consequently, both pCO_2 and CO_2 concentration decrease.

Patra et al. (1993) measured the pH of body fluids from sheep engorged with grain. Ruminal pH reached its lowest point 12 hours post-feeding whereas blood pH was lowest at 24 hours; urine and cerebrospinal fluid reached minima at 48 hours post-feeding. Within 12 hours post-feeding, lactic acid concentrations had increased in rumen fluid, blood and cerebrospinal fluid. Uhart and Carroll (1967) also concluded that urine acidity was greater for steers that were fed a 90% grain diet without being adapted. Blood pH and ruminal pH in glucose-induced acidotic cattle steadily decreased through 12 hours post-engorgement in a study by Nagaraja et al. (1982). Nagaraja et al. (1982) detected small increases in concentrations of L(+) and D(-) blood lactate during 12 hours post-engorgement.

For clinical diagnosis of acidosis, blood is the index parameter. When blood pH declines below 7.35, acidosis is considered present. With ruminal

acidosis, packed cell volume (PCV) of blood increases. This is because plasma fluids move into the rumen from the plasma when osmolality of ruminal contents exceeds that of blood. Ultimately, tissues become dehydrated as a large amount of water is pulled from blood.

Osmotic Pressures. Under normal conditions, ruminal osmotic pressure ranges from 240 to 265 mOsm/L with roughage diets and from 280 to 300 with concentrate diets (Garza et al., 1989). However, with acidotic conditions, ruminal osmolality can reach 500 mOsm/L (Carter and Grovum, 1990). Although lactate is the major constituent behind the increase in ruminal osmotic pressure (Huber, 1976 and Howard, 1981), glucose and VFA also contribute to the increased osmolality. This elevated osmolality inhibits VFA absorption; this exacerbates acid and pH problems within the rumen. In addition, when ruminal osmolality exceeds 350 mOsm/L, ruminal contents may become stagnant because of bacterial inhibition (Carter and Grovum, 1990). Movement of water rushing into the digestive tract to maintain osmotic pressure leads to increased water consumption and diarrhea of acidotic animals. Huber (1971) stated that body water content (plasma, interstitial and intercellular fluid) was reduced by 8% due to high ruminal osmolality during acidosis in sheep.

Additional damage can occur during acidosis. With acidosis and the high osmotic pressure, the epithelial tissue of the rumen is damaged; areas can be totally destroyed in extreme cases. A damaged ruminal epithelium permits ruminal bacteria to invade the body; sepsis from ruminal microbes is responsible for liver abscesses, a large economical concern in the feedlot cattle industry because liver makes up approximately 2% of carcass weight (Tindall, 1983). In addition cattle with severe abscesses generally have slower and less efficient gains.

Glucose. Glucose, though an important intermediate of bacterial metabolism in the rumen, normally is present only at extremely low concentrations. Indeed, with hay-fed ruminants, glucose concentrations in the rumen usually are below the limit of detection. But in wheat-fed acidotic sheep,

glucose reached concentrations of 13.5 mM (Ryan, 1964b). This compares with plasma glucose concentrations of 3 to 4 mM. Horn et al. (1979), in a grain engorgement study, detected ruminal glucose concentrations over 9 mM. Ryan (1964a) determined that ruminal concentrations of glucose increased with grain intake and that high ruminal glucose concentrations provided an ideal milieu for establishment of organisms responsible for acidosis. Slyter (1976) suggested that with a decreased ruminal pH, free amylase increases while utilization of glucose by ruminal microbes decreases. When glucose production increases and utilization rates decrease, glucose accumulates in the rumen and the number of glucose-using lactic acid-producing bacteria will increase. Presence of free glucose also inhibits lactic acid metabolism within the rumen (Slyter, 1976). Leedle et al. (1995) found that ruminal glucose concentrations increased nearly eightfold when diet concentrate was increased to 90%.

Rumen Motility. Huber (1976) determined that when ruminal pH fell to 5.0, amplitude and frequency of ruminal contractions progressively diminished and eventually stopped. Bruce and Huber (1973) proposed that intestinal hormones help to regulate rumen and intestinal motility during an acid overload in the rumen. Kezar and Church (1979) also found that motility in the reticulo-rumen in sheep was greatly reduced 4 to 10 hours after a large dose of sucrose was introduced into the rumen. Consequently, some depression in ruminal motility is expected during acidosis.

Endotoxins. With an acidotic rumen and a changed ruminal flora, Mullenax et al. (1966) proposed that bacterial endotoxins, e.g., lipopolysaccharide endotoxin, released by lysing gram-negative ruminal bacteria, are absorbed through the wall of the rumen. Mullenax et al. (1966) found that intravenous injection of the endotoxin caused ruminal motility to decrease or cease. Endotoxins from coliforms and clostridia also may contribute to other acidotic symptoms including diarrhea.

Histamine. Laminitis and hoof damage have been attributed to elevated blood concentrations of histamine (Vermunt and Greenough, 1994). In the

rumen of engorged sheep, Dain et al. (1955) found that histamine concentrations exceeded 70 ug/ml of ruminal ingesta and that histamine concentration was correlated inversely with ruminal pH. Histamine formation was apparent when pH of the rumen dropped below 5.0. Sanford (1963) suggested that histamine concentration in the rumen increased as pH decreased because histidine and other amino acids in ruminal fluid were deaminated. Under normal conditions, histamine is not extensively decarboxylated in the rumen, but when ruminants consume an excessive amount of carbohydrate, ruminal histamine concentration increases considerably. Rodwell (1953) isolated one species of lactobacillus that produced histidine decarboxylase and decarboxylated histidine to histamine at a low pH.

Conventional Management of Acidosis

Many symptoms of acidosis are apparent in ruminants given access to readily-fermented carbohydrates. These include: marked reduction or cessation of appetite; loose feces or diarrhea; listless, depressed or distressed appearance; excessive water consumption; founder or sore feet; kicking or licking at sides; increased respiration; and death (Elam, 1976; Kezar and Church, 1979). Other observations used in appraising acidosis include: decreased rate of gain and feed efficiency; liver abscesses; rumenitis; and an altered blood profile. Because acidosis of feedlot cattle continues to be a prominent production problem, many methods to reduce or alleviate acidosis have been studied. Methods being researched currently by various workers include narrow-spectrum antibiotics (e.g., ionophores, virginiamycin), dietary buffers (e.g., bicarbonates), and direct-fed microbial preparations (e.g., lactobacilli). Grain processing and feeding management to decrease the acid load also can help reduce the incidence of acidosis.

Narrow-spectrum antibiotics routinely are incorporated into ruminant diets to improve efficiency of production. Those used most commonly are the antibiotic ionophores - monensin and lasalocid. Other ionophores include laidlomycin propionate, tetronasin, and salinomycin. These ionophores usually

improve feed efficiency of feedlot cattle. Through enhancing ruminal propionate production, lasalocid and monensin may decrease lactate production, decrease methane loss, and reduce proteolysis and deamination (Bergen and Bates, 1984; Schelling, 1984). Ionophores appear to reduce the incidence of acidosis either because of alterations in ruminal flora or fermentation or reduce meal size.

Prevalent among the gram-positive lactic acid producing bacteria are *Streptococcus bovis* and *Lactobacillus* species (Slyter, 1976). Ionophores like monensin, being active against gram-positive bacteria, inhibit ruminal production of lactic acid (Nagaraja et al., 1986). Monensin also inhibits growth of *Streptococcus bovis* and *Lactobacillus* species (Dennis et al., 1981) and lasalocid and monensin effectively prevented induced lactic acidosis in cattle (Nagaraja et al., 1981). Although both ionophores were effective in maintaining a higher ruminal pH and prevented accumulation of lactate, lasalocid seemed more potent at a dose of 0.65 mg/kg body weight (Nagaraja et al., 1982). In an in vitro study conducted by Tung and Kung (1993), monensin prevented lactate accumulation and pH remained higher than in untreated cultures. Molar proportions of propionate were more than 40% greater and acetate 15% less in treated than untreated cultures.

In contrast to lasalocid and monensin, laidlomycin propionate has not been shown to prevent acidosis although it may reduce the severity of acidosis during adaptation to a high concentrate diet (Baur et al., 1995). Ruminal concentrations of D-lactate were reduced by 6 but not 12 mg/kg of laidlomycin propionate (Galyean et al., 1992). However, Galyean et al. (1992) detected no effects of laidlomycin on ruminal pH or concentrations or proportions of ruminal VFA.

Thiopeptin, a selective antibiotic but not an ionophore, may like ionophores increase rate and efficiency of gain (Gill et al., 1979). At a dose of at least 1.5 mg/kg body weight, thiopeptin prevented induced lactic acidosis in cattle (Muir et al., 1981; Nagaraja et al., 1982). Muir et al. (1980) found that acidosis was controlled in lambs fed thiopeptin at 22 ppm with lactate

concentration being 68% lower when thiopeptin was fed. Kezar and Church (1979) detected lower concentrations of lactic acid (<30 mM) and higher ruminal pH with thiopeptin feeding together with an increase in propionic acid and a decrease in the acetate to propionate ratio.

Tetronasin, an ionophore, has been shown to increase gain and efficiency of cattle (Davies, 1982). In vitro, tetronasin prevented accumulation of lactic acid and maintained a non-lactate producing bacterial environment when added at the same time as an excess of glucose (Newbold and Wallace, 1988). When addition of tetranasin was delayed until 24 hours after glucose was dosed, the proliferation of lactobacilli was reversed, lactate concentrations dropped, and culture pH rose. In contrast, monensin failed to suppress the growth of lactobacilli when added to a culture 24 hours after glucose was added. *Lactobacillus casei* is 55 times more sensitive to tetronasin than to monensin (Newbold et al., 1988). Salinomycin also has been found to prevent induced acidosis in cattle. In fact, salinomycin appears to be at least 3 times more potent than either lasalocid or monensin (Nagaraja et al., 1985).

Another clinical management tool for acidosis that has been tested but has not produced consistent benefits is feeding of dietary buffers. This inconsistency probably relates to the long lag time (6 to 10 hours) between the time a meal is consumed and time that ruminal pH reaches a minimum. Certainly, sodium bicarbonate aids in neutralizing ruminal acids (Prasad and Rekib, 1975; Shuey, 1993) but usefulness in preventing rather than treating acidosis seems doubtful. Horn et al. (1979) found that dietary buffers such as 2% sodium bentonite, 1% sodium bentonite plus 1% dolomite, and 1% sodium bentonite plus 1% potassium bicarbonate helped to maintain higher ruminal pH at 4 and 8 hours post-feeding in dosed than in control steers. However, ruminal glucose and total VFA concentrations and osmolalities were not affected by including these buffers in the diet.

Direct - Fed Microbes

With the growing public concern among the consumers of beef and dairy products over abuse of antibiotics and growth stimulants in the animal feed industry, interest in the effects of direct-fed microbial (DFM) on animal performance has blossomed. DFM can have favorable ruminal effects. Burroughs et al. (1960) reported that feedlot beef cattle fed DFM gained 7% faster. Of specific concern with DFM are the specific organism(s) involved and their viability and metabolism, dosage rate, ruminal establishment, and site of establishment.

Aspergillus oryzae and *Saccharomyces cerevisae* are two microbial products that have been tested quite extensively. Van Horn et al. (1984) fed *A. oryzae* to lactating dairy cows and detected significant increases in digestibility of dry matter and acid detergent fiber (ADF) but no differences in feed intake or milk production. In contrast, Gomez-Alarcon et al. (1991) detected an increase in milk production of cows dosed with *A. oryzae* during early lactation. Increases in digestibility of dry matter, crude protein and hemicellulose have been reported with non-lactating cows (Weidmeier et al., 1987). Weidmeier et al. (1987) found with non-lactating cows that feeding *S. cercvisiae* had increased total tract digestibility for crude protein and hemicellulose but not for dry matter or ADF. When *S. cercvisiae* and *A. oryzae* were fed together, total tract digestibility of dry matter, crude protein and hemicellulose all were increased.

Erdman et al. (1989) detected no effect of feeding *S. cercvisiae* on dry matter intake, milk production, or milk composition in mid-lactation. In contrast, Williams et al. (1991) found that cows fed *S. cercvisiae* produced more milk due to higher feed intake during early lactation. When cows were fed a diet consisting of corn silage, grain, and hay, Wohlt et al. (1991) found that the cows fed *S. cercvisiae* had higher dry matter intakes and milk production during early lactation.

Harrison et al. (1988) fed *S. cercvisiae* to lactating cows. Ruminal pH, acetate concentration, and molar acetate to propionate ratio all were lower but

molar proportions of propionate and valerate were increased. Adams et al. (1981) fed *S. cercvisiae* to steers and wethers. Although neither ruminal fermentation nor digestibility was changed, intake was increased significantly.

Counotte et al. (1983) reported that one bacterial species, *Megashaera elsdenii,* was responsible for converting more than 80% of the lactate formed in the rumen to VFA. In recent years researchers have tried to use *Megashaera elsdenii* as a direct-fed microbe to prevent the accumulation of lactate in ruminants. Inoculation with in vitro cultures with *M. elsdenii* prevented accumulation of lactic acid and an excessive drop in pH (Kung and Hession, 1995). *M. elsdenii* is unique; it produces propionate from lactate but does not form propionate from glucose (Marounek et al., 1989).

Interest by the scientific community has increased recently in manipulating the ruminal microflora to enhance feedstuff utilization and alleviate metabolic disorders. By stabilizing ruminal pH through enhancing lactate use without using antibiotics or ionophores may improve performance through overcoming the economic loss associated with acidosis. Other disorders such as "nitrate" poisoning can be prevented by supplementing a diet with *Propionbacterium acidipropionici* (P5) (Swartzlander, 1994). Certain strains of propionibacteria studied by Parrott (1996) were effective for preventing lactate accumulation and retard declines in pH in vitro.

Propionibacteria

The genus *Propionibacterium* has been well characterized by Hettinga and Reinbold (1972a, b, and c). Propionibacteria are gram-positive, rod-shaped, nonspore forming, nonmotile, facultative anaerobic bacteria. The *Propionibacterium* genus produce primarily propionic acid and acetic acid as fermentation end-products. Although they can grow between 5 °C and 40 °C, growth is most rapid at 30-37 °C (Cummins and Johnson, 1986). Propionibacterium have an optimal pH for growth, between 6.5 and 7.0 (Tittsler, 1940) although the optimum range for different species ranged from 4.6 to 8.5.

Propionibacteria are used routinely for making Swiss cheese. After a primary culture ferments lactose to lactate, propionibacteria serve as the secondary culture to ferment lactate to propionate, acetate and CO_2 during cheese ripening (Hettinga and Reinbold, 1972b; Langsrud and Reinbold, 1973) giving Swiss cheese its characteristic myopic eyes and flavor (Reinbold, 1985). The four recognized dairy-related species of the genus *Propionibacterium* includes *P. freudenreichii, P. acidi-propionici, P. theonii,* and *P. jensenii* (Reinbold, 1985).

Less favorable species include *P. acnes, P. avidum, P. granulosum,* and *P. lymphophilum* (Reinbold, 1985). These four species have been found in such habitats as abscesses, blood, pus and wounds. Of the four dairy-related species *P. jensenii* is the only one found in these habitats.

Other industrial applications for the genus *Propionibacterium* have been explored in recent years. Kornyeva (1981) and Nabukhotny et al. (1983) used propionibacteria as a direct-fed microbial product for humans. They also are important as a direct-fed microbe in beef cattle to prevent "nitrate" toxicity (Swartzlander, 1994). Because of their ability to produce Vitamin B_{12} and propionic acid, the genus *Propionibacterium* has been used for industrial production of these products (Perlman, 1978; Playne, 1985). Propionibacteria also has found use as an inoculant for grain and silage (Woolford, 1975; Flores-Galarza et al., 1985).

Based on their ability to utilize lactate and grow at a low pH, species of propionibacteria recently have been studied as direct-fed microbes to aid in the prevention of ruminal acidosis. Parrott (1996) determined that certain select strains of propionibacteria increase the lag time before lactic acid accumulated and suppressed the rate at which H^+ concentration increased in ruminal fluid in vitro.

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CHAPTER III.

ESTABLISHMENT AND RUMINAL EFFECTS OF A PROPIONIBACTERIA CO-CULTURE DURING ADAPTATION OF HEIFERS TO A HIGH CONCENTRATE DIET

Abstract

The objectives of this study were to determine if a propionibacteria co-culture could be established in the rumen and its effects during a 21 d study. Three ruminally cannulated heifers (500 kg) were switched from an all forage to a 90% concentrate diet in 9 days. Each heifer was inoculated with a propionibacteria co-culture which included three strains (81, 89, and 104). Each animal received 10 mL of a liquid culture containing 2 x 10^{11} cfu/mL of each strain daily. Ruminal fluid pH, lactate, volatile fatty acid (VFA) and glucose concentrations and ruminal osmolality were monitored. To examine ruminal establishment, propionibacteria populations in ruminal fluid enumerated. During the 9-day adaptation period, acidosis never occurred; ruminal pH remained above 5.4 and lactate never exceeded 3.30 mM. However, three bouts of acidosis occurred during the following 12 d. Numbers of propionibacteria in ruminal liquid never exceeded those expected from the dosing alone and were not detectable 7 days after inoculation ceased. This indicates that these three strains never became permanently established within the rumen. Yet, because ruminal lactate never accumulated during the acidosis challenge, inoculation may have prevented lactate accumulation within the rumen.

(Key words: Propionibacteria, Cattle, Acidosis)

Introduction

Certain strains of propionibacteria can reduce lactate accumulation and prevent severe drops in pH of ruminal fluid during in vitro incubation (Parrott,

1996). Based on the results of screening of 44 strains by Parrott (1996), three strains of propionibacteria (81, 89, and 104) were chosen to be used together in an attempt to prevent acidosis in cattle being rapidly adapted to a high concentrate diet. Typical adaptation periods in the feedlot industry range from 21 to 28 days. Shortening this adaptation period by 12 to 19 days would reduce the total number of days that feedlot cattle must be fed before marketing. The objectives of this study were to examine the survival of three strains of *Propionibacterium* that were inoculated and to monitor effects of inoculation on pH and lactate concentration in the rumen of cattle being adapted rapidly to a high concentrate diet.

Materials and Methods

Three adult ruminally cannulated heifers (500 kg initially) were used in this 21 day experiment. These heifers were housed in individual metabolism stalls under controlled lighting and temperature conditions. Each heifer was inoculated each day of the 21 day experiment by dosing with 10 mL of microbes containing 2×10^{11} cfu/mL of each strain of *Propionibacterium* 81, 89, and 104 directly into the rumen at 0800.

During the first 9 days of this 20 day experiment, the diet for these three heifers was changed from a 100% grass hay to 90% concentrate diet. Composition of the various concentrate diets is shown in Tables 1 and 2. On day 0, each heifer was fed grass hay; on days 1 and 2, the diet contained 30% concentrate. Thereafter, the percentage of concentrate in the diet was increased by 15% every second day as shown in Table 3. Each heifer had ad libitum access to its diet and unlimited access to water.

Intake was monitored daily during the 21 day experiment. Ruminal pH, lactate and VFA concentrations were measured four times each day (0, 2, 6, and 10 hr after fresh feed was provided) during days 0 through 10 and once daily (at 0 hr postfeeding) on days 11 through 21. Ruminal glucose concentrations and osmotic pressures were measured once on days 0 through 3, 6 through 10, and on days 15, 17 and 21. Ruminal fluid samples were taken on days 0 through 10,

14, 17, and 21 at 1000 (2 hr after each inoculation) and plated on a selective agar at dilutions of $1x10^{-2}$, $1x10^{-3}$, $1x10^{-4}$, and $1x10^{-5}$ to appraise the number of free floating propionibacteria present in the rumen.

At each sampling time, approximately 50 mL of ruminal fluid was removed using a vacuum pump connected to in-dwelling ruminal filters. Ruminal pH was measured immediately using a portable Digi-Sense^{R} LCD pH meter (Cole Parmer Instrument Co., Chicago, IL). A 3 mL sample of fluid was transferred to a microcentrifuge tube; debris was pelleted by centrifugation at 5500 X g for 10 min. The supernatant was decanted and frozen (-20^{O} C). At a later time, these samples were thawed and assayed for lactate and VFA using a Hewlett Packard 1090 High Pressure Liquid Chromatography (HPLC) system equipped with a diode-array detector (Hewlett Packard, Atlanta, GA). Before analysis, the samples were defrosted slowly; 0.5 mL of the supernatant fluid was transferred to a second tube and acidified with an equal volume of 0.01M sulfuric acid solution to prevent fermentation. These acidified samples were filtered through 0.2 um filters directly into 2 mL HPLC autosampler vials and capped; subsequently, these samples were injected into a 0.005 M H_2SO_4 mobile phase heated to 65^{O} C and separated using a Bio Rad HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA). Areas of peaks, detected with a diode array detector at 210 nm, were used to calculate compound concentrations by comparison with external standards eluted at similar times.

The remaining ruminal fluid that was collected was transferred on ice to the laboratory for plating. When ruminal glucose concentrations and osmotic pressures also were being analyzed, an additional 50 mL of ruminal fluid was withdrawn from the rumen. This fluid also was held on ice until being centrifuged at 5500 X g for 10 min. to remove solid debris. The supernatant fluid was used for osmotic pressure and glucose analysis. Ruminal osmolalities were measured using an Osmette Precision 2007 osmometer (Precision Systems, Inc., Sudbury, MA) using 500 UL samples. Glucose concentrations were determined using a procedure adapted from the Rapid Stat ^{TM} Kit and the Glucose Rapid Stat

Reagent (Pierce Chemical Co., Rockford, IL) using a single reagent, o-toluidine at 6% in glacial acetic acid, for the quantitative colorimetric determination of glucose. In the presence of heat and acid, o-toluidine reacts readily with glucose to form a blue-green complex. The intensity of the color formed, measured with a Gilford Response UV-VIS spectrophotometer (Gilford Instrumentation, Oberlin, OH) at 630 nm, was proportional to the glucose concentration.

Results and Discussion

Dry matter intake fluctuated noticeably as the heifers were being adapted to their high concentrate diet (Table 4) with DM intake declining considerably as the concentrate percentage was increased. Figure 1 shows the relationship between ruminal pH and intake; whenever ruminal pH declined, feed intake typically decreased the subsequent day. High ruminal osmotic pressure also may depress feed intake (Carter and Grovum, 1990). Even though ruminal osmolalities were not measured each day, increases in ruminal osmotic pressure to levels above 340 mOsm appeared to decrease feed intake (Figure 2). Compared with expected osmolalities of 280 to 300 mOsm with concentrate diets (Garza et al., 1989), all values were quite high during diet adaptation.

During the 9 day adaptation period, all three animals maintained a ruminal pH above 5.4. In most cases, ruminal pH remained within the normal range of 5.5 to 7.0 (Annison and Lewis, 1959). This was impressive considering that acidosis would be expected in animals subjected to such an abrupt dietary change. Perhaps the co-culture of propionibacteria that each animal received aided in the maintenance of a "normal" ruminal pH although this is impossible to determine because no control animals were included in this experiment. Lactate never accumulated during the 9 d adaptation period. Parrott (1996) previously found that the strains being inoculated, *Propionibacterium* strains 81, 89, and 104, prevented lactic acid from accumulating in vitro.

Lactic acid, particularly D-lactic acid, has been implicated as the culprit in ruminal acidosis. However, lactic acid cannot be the sole factor involved in acidosis because VFA also can accumulate and depress ruminal pH (Goad,

1990). During adaptation of these heifers to their high concentrate diet, total VFA concentration within the rumen increased from a mean of 14.9 mM for the heifers on day 0 to a mean of 292.3 mM on day 10. However, ruminal pH began to decline even before VFA accumulation began, so ruminal pH depression was correlated imperfectly with total VFA concentration (Table 5).

Three acidosis incidents occurred; each occurred after the 9 d period of diet adaptation. Two of the three animals exhibited ruminal pH values below 4.8, a pH value consistent with diagnosis of ruminal acidosis (Slyter, 1976). These three incidences are described below.

On day 10 of the trial, animal 911 had a mean ruminal pH of 5.42. However, ruminal pH declined throughout d 10. Six hr after being fed (1400), ruminal pH reached 5.1 with 17.76 mM of lactic acid; by 14 hr, pH reached 4.8. At 14 hr post-feeding (2200), lactic acid concentration was 55.5 mM, considerably higher than the 1 to 4 mM concentrations normally found within the rumen of cattle fed concentrate diets. Uhart and Carroll (1967) reported that lactic acid had reached 99.96 mM when ruminal pH declined to 4.80. Dunlop (1972) found lactate concentrations up to 150 mM in sheep force-fed wheat. At 2200, we dosed this heifer via ruminal cannula with sodium bicarbonate to prevent rumen wall damage and blood acidosis. Ruminal pH was back to neutral conditions (pH = 7.1) on d 11. On day 9, when the 90% concentrate diet was first introduced, and the day prior to the acidosis incident, heifer 911 consumed a very large amount of feed (20.2 kg); this probably contributed to the decline in ruminal pH observed on d 10. Ruminal concentration of glucose and ruminal osmotic pressure were increased during d 10 when acidosis occurred. Glucose increased from unobservable quantities on d 9 to 1.68 mM on d 10; ruminal osmotic pressure increased from 319 to 374 mOsm/kg. Slyter (1976) and Krehbiel et al. (1995) detected increases in ruminal glucose concentration when ruminal fluid pH dropped.

On d 19 of the trial, heifer 911 again had a low ruminal pH (4.7). The night before this incident, this heifer had lost the cap to her ruminal cannula plus

a large amount of ruminal fluid. Lactate was present at 21.09 mM. Even though no treatment was administered, ruminal pH subsequently increased to 5.6 by d 21 with no lactate present. Briggs et al. (1957) observed that lactic acid concentrations in sheep greater than 20 mM always were associated with ruminal pH values below 5.0. When the quantity of fluid in the rumen is low, from either fasting or loss through the cannula, the quantity of buffered ruminal contents to equilibrate with newly consumed feed is reduced; this may enhance the potential for acidosis.

Animal 528 had a bout of acidosis on d 15; ruminal pH on d 15 was 4.8 and lactic acid was 20.6 mM. This concentration of lactic acid was lower than found by Kezar and Church (1979) at this ruminal pH with 34.2 mM at 4.73. Approximately 9 h post-feeding, ruminal pH had declined to 4.6. Sodium bicarbonate was administered and ruminal pH increased to 6.3 on d 16 with no lactate being present. Glucose concentration in the ruminal fluid during d 15 was 3.66 uM while ruminal osmotic pressure was 513 mOsm/kg. Intake during d 15, at 1.4 kg, was markedly depressed from d 14 when this heifer consumed 13.6 kg of feed. Even prior to the acidosis incident on d 15, lactate concentrations had begun to climb, being 28.2 mM on d 13 and 13.2 mM on d 14.

The remaining animal, 531, exhibited only minor ruminal pH declines to 5.4 throughout the entire 21 days on trial. This heifer never had ruminal lactate concentrations that were detectable.

Contrary to expectations and literature information, these acidosis events occurred after, not during, the nine day adaptation. Furthermore, in all instances, concentrations of lactic acid analysis of rumen fluid samples remained relatively low. This indicates that acidity was largely the result of accumulation of acids other than lactic acid. These observations support the theory that inoculation may have been inhibiting lactic acid accumulation. Each case of acidosis was preceded by spikes in feed intake, high ruminal osmolality, and high ruminal glucose concentrations.

Table 6 shows the rumen populations of propionibacteria for the three animals during the experiment. The daily dosage was 2 X 10^{11} cfu. If ruminal volume was 18% of body weight as predicted from the equation: Ruminal volume = 12.8 + 0.49(concentrate intake) + 2.25(roughage intake), developed by Owens and Goetsch (1988), this means that each day, 3.7 X 10^6 cfu were added to each mL of ruminal contents. Assuming that dosed microbes flowed out of the rumen with liquids and that ruminal dilution rate also was as predicted from the equation: Liquid dilution rate = 4.21 + 0.77(concentrate intake) + 2.32(roughage intake) developed by Owens and Goetsch (1988), expected ruminal concentrations can be predicted. All counts were taken 2 h after the last dosing. If the inoculum was fluid-borne and liquid dilution rate was 5.1%/h, then the expected concentration at 2 h was 3.3 X 10^6. Because bacterial counts on days 0 through 7 are questionable due to concerns about plate contamination, only values for d 8, 9, 10, 14, 17 and 21 are reported. Note that concentrations of propionibacteria strains never reached a concentration as high as would be expected from inoculation alone. This suggests that these particular strains may be invading the pilli of the rumen wall rather than free floating. Another possibility may be that the strains are being washed out from the rumen with the fluid. Ruminal concentrations match those reported previously (1 x 10^5 cfu/mL) of propionibacteria dosed at a rate of 1 x 10^7 cfu per animal daily and plated 6 hr after inoculation (Swartzlander, 1990). Ruminal samples also were taken from these heifers 7, 14 and 21 d after dosing with the culture had been discontinued. No propionibacteria colonies were detected in any heifer at any of these sampling times. These results indicate that these strains were not permanently established within the rumen or may have invaded the rumen pilli. After this measurement, the rumen walls of these heifers were swabbed and plated at previous dilutions. However, no colonies were detected. Therefore, we conclude that no permanent establishment was made. In contrast, Swartzlander (1990) reported that in forage-fed cattle, Propionibacteria strain P5 became

permanently established within the rumen, still being detectable 115 d after the last previous inoculation at a concentration of 1 X 10^7 cfu/mL ruminal liquid.

Implications

During ruminal inoculation with *Propionibacterium* strains 81, 89, and 104, concentrations in the rumen reached but never exceeded the concentrations expected. Without permanent establishment, of these selected strains of propionibacteria, such cultures would need to be provided each day to each animal to be effective. However, because ruminal lactate never accumulated beyond background concentrations, the inoculation may have aided in the prevention of lactate accumulation. In three cases, ruminal pH dropped below 5.0, indicative of acidosis. Further experiments are needed to compare effects noted in this study with those of animals that do not receive propionibacteria cultures.

Table 1. Composition of concentrate diets (DM basis).

	Concentrate content				
Ingredient, %	30%	45%	60%	75%	90%
Ground corn	22.1	36.9	51.8	66.9	82.0
Cottonseed hulls	35.1	26.2	17.7	7.6	0.0
Alfalfa pellets	35.1	29.2	22.7	17.7	10.2
B-075 Supplement	7.7	7.7	7.8	7.8	7.8

Table 2. Composition of B-075 supplement on a DM basis.

Ingredient, %	
Cane molasses	2.37
Limestone 38%	14.26
Salt	3.79
Vitamin A-30,000	0.13
Cottonseed meal	59.22
Soybean meal 44%	11.72
Manganous oxide	0.08
Zinc oxide	0.06
Potassium chloride	1.93
Urea	6.44

Table 3. Nine day adaptation scheme.

Day	% Concentrate
0	0
1	30
2	30
3	45
4	45
5	60
6	60
7	75
8	75
9-21	90

Table 4. Mean intakes during the adaptation period.

Day	% Concentrate	Mean DM intake (kg.)
1	30	22.7
2	30	10.0
3	45	11.0
4	45	14.0
5	60	12.3
6	60	8.9
7	75	11.8
8	75	10.3
9	90	13.6

Figure 1. Mean ruminal pH vs. mean daily intakes.

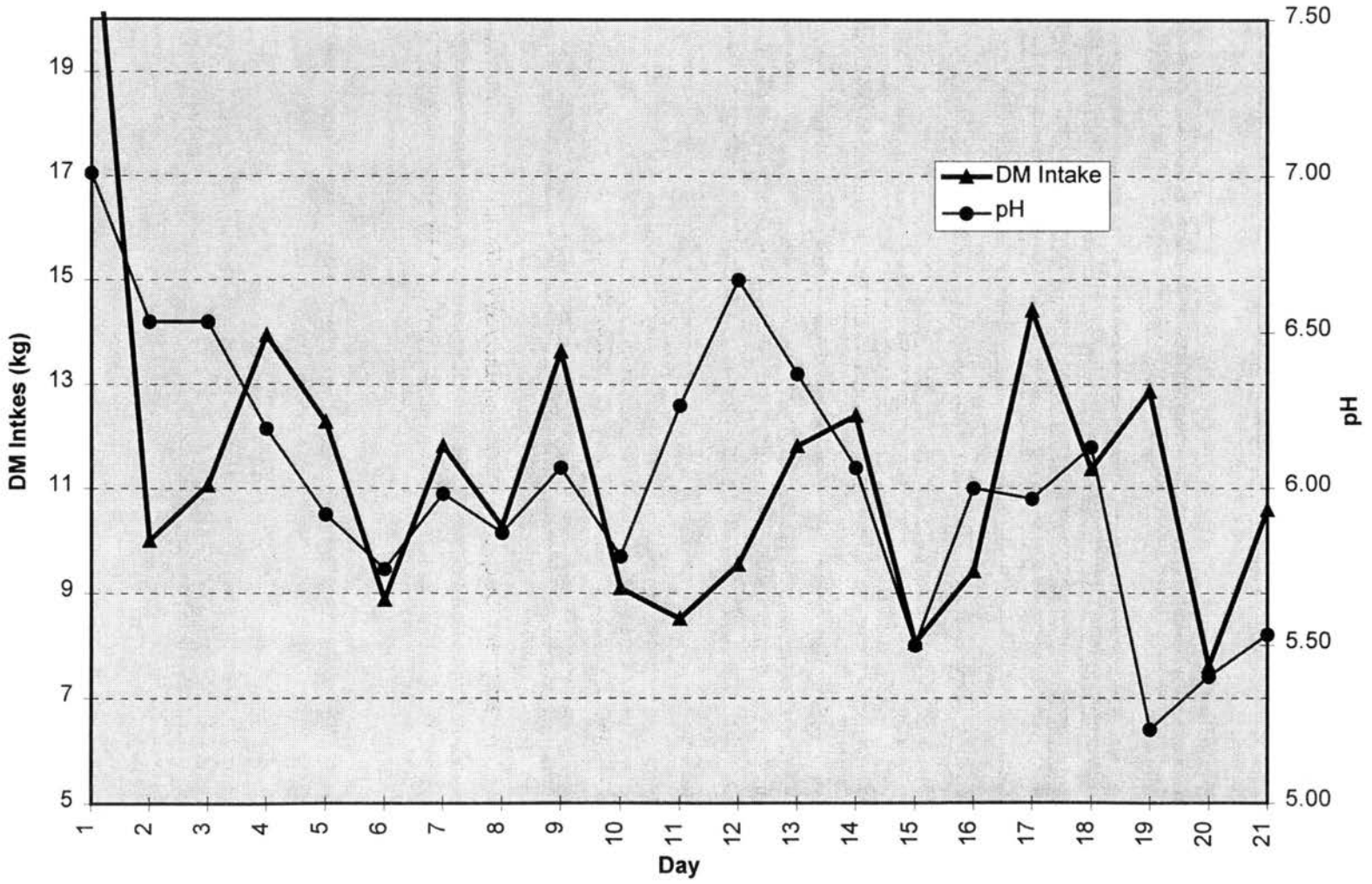

Figure 2. Mean ruminal osmotic pressures when compared to mean daily intakes.

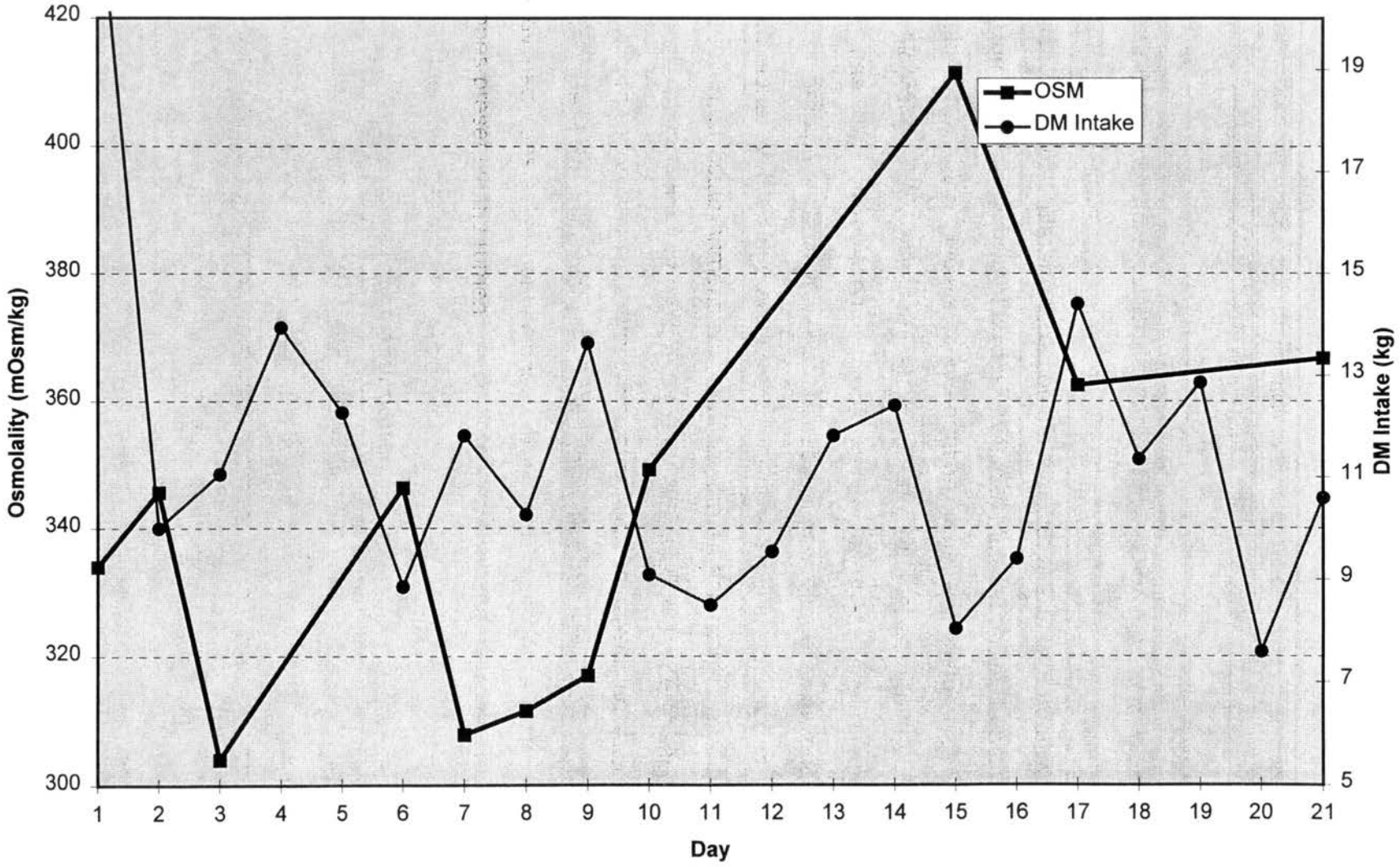

Table 5. Daily mean ruminal pH and total VFA concentrations.

Day	pH	[VFA] (mM/L)
0	7.14	14.9
1	7.01	32.0
2	6.53	33.3
3	6.53	35.7
4	6.19	44.3
5	5.92	44.6
6	5.74	132.3
7	5.98	46.3
8	5.86	152.4
9	6.07	191.4
10	5.78	292.3
11	6.27	122.0
12	6.67	89.5
13	6.37	142.0
14	6.07	159.4
15	5.50	166.5
16	6.00	132.1
17	5.97	169.0
18	6.13	116.4
19	5.23	82.3
20	5.40	97.0
21	5.53	78.6

Table 6. Survival of the *Propionibacterium* co-culture (81, 89, and 104) in the rumen .

Day	*Propionibacterium* (cfu/mL)
8	2.2×10^5
9	2.2×10^5
10	3.0×10^5
14	8.7×10^5
17	4.1×10^5
21	3.2×10^5

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CHAPTER IV.

EFFECTS OF INOCULATION OF CATTLE BEING ADAPTED TO CONCENTRATE DIETS WITH PROPIONIBACTERIA CO-CULTURES

Abstract

Three trials were conducted to determine how ruminal inoculation with a propionibacteria co-culture influenced acidosis conditions associated with rapid adaptation to a high concentrate diet. In trial 1, 8 steers (400 kg) were used. A 3-way propionibacteria co-culture (strains 81, 89, and 104) was inoculated at a rate of 6 X 10^{11} cfu daily into the rumen of 4 of these steers. Composition of the diet for these steers was gradually switched from 100% roughage to 90% concentrate in either 5 or 9 days. Neither inoculation nor adaptation time altered ($P>0.05$) ruminal pH, ruminal concentrations of pH, lactate, VFA, or glucose or osmotic pressure. No effects of inoculation on blood parameters (pH, bicarbonate, and packed cell volume) were detected; during the 5 and 9 d adaptation periods no cases of acidosis were detected. However, following adaptation (d 8 to 21), low ruminal pH (< 5.3) was indicative of acidosis in three calves. Despite these low ruminal pH values, lactic acid never accumulated to concentrations above 20.87 mM. In trial 2, 8 heifers (438 kg) were adapted from a roughage to a concentrate diet in only 5 days. Four of these heifers were ruminally inoculated daily with 1.3 x 10^{12} cfu of a mixture of 5 different propionibacteria strains (41, 63, 81, 89, and 104). Although ruminal pH values were not significantly different between inoculated and non-inoculated calves, inoculated cattle tended to have a higher ruminal pH for the first 9 days of the trial, dropping by 1.29 and 1.46 pH units for the inoculated and the control cattle, respectively. At some time during this trial, each calf exhibited acidic ruminal pH values although lactic acid accumulated in the rumen above a concentration of

15 mM in only one of these 8 cases in which ruminal pH fell below 4.7. Inoculation had no significant effect on either ruminal (glucose and VFA concentration; osmotic pressure) or blood measurements (pH; bicarbonate; packed cell volume; glucose; osmolality). In trial 3, 14 cattle (437 kg) were used. Seven of these were inoculated (as in trial 2) with the 5 strain inoculum 14 days prior to an abrupt switch in diet composition from 50% to 90% concentrate. After 1 d on the 90% diet, feed was withdrawn for 24 h; for the next 2 days, cattle were force fed (via ruminal cannula) the 90% concentrate diet at 2.5 % of body weight each day. Ruminal pH indicative of acidosis was detected in both inoculated and control cattle. But again, lactate concentration remained below 20 mM. Inoculation did not ($P>0.05$) alter ruminal pH or other ruminal parameters (VFA, lactate, glucose, and osmolality). During each of these trials, ruminal populations of propionibacteria never exceeded concentrations expected from the inoculum alone. Interactions among the individual strains, detected in vitro, may have prevented establishment and reduced the antacid activities of these cultures. However, the fact that ruminal pH dropped very low in the absence of lactic acid accumulation suggests that inoculation with lactate-using microbes maybe moot. Selective microbial plating indicated that certain cattle in these trials already had substantial populations ($> 1.21 \times 10^8$) of native lactic acid-using microbes present in the rumen.

(Key words: Propionibacteria, Cattle, Acidosis)

Introduction

Most cattle entering a feedlot are abruptly challenged with a diet change; accustomed to a forage-based diet, they must adapt rapidly to consume diets based on grain. During conversion from forage-rich to concentrate diets, ruminal acidosis often occurs (Brent, 1976; Leedle et al., 1995). To reduce the incidence of acidosis, the time allocated for adaptation often is prolonged to 21 to 28 days. Decreasing the length of this adaptation period would decrease the number of days that cattle must be fed prior to marketing. Inoculation with propionibacteria that can prevent ruminal lactate accumulation and severe drops in ruminal pH

(Parrott, 1996) may reduce the time needed for ruminal adaptation to concentrate diets. The objective of these trials was to determine the effectiveness of a propionibacteria co-culture for preventing ruminal acidosis during rapid adaptation of cattle from a roughage to a concentrate diet.

Materials and Methods

All cattle used in these trials were surgically-fitted with rumen cannulas. In trials 1 and 2, cattle also were equipped with indwelling jugular catheters for blood sampling.

Trial 1. Eight yearling steers (400 kg) were used in this trial; adaptation time from a roughage to a concentrate diet was 5 days for four of these steers and 9 days for the other four steers as listed in Table 7. Two of the steers on each adaptation scheme were inoculated intraruminally at 0800 each day with a 3 strain (81, 89, and 104) propionibacteria co-culture. Each steer was dosed with 10 mL of a solution containing 2 X 10^{11} cfu/mL of each strain of *Propionibacterium*. Inoculation began on day 1 of adaptation. This trial lasted 21 days. Diet compositions are shown in Table 8. Each steer received 13.6 kg of DM once each day at 0800; this is equal to 3.4% of mean body weight. Total feed intake was measured daily.

Ruminal pH, lactate and VFA concentrations were measured 6 times each day (0, 4, 8, 10, 12, and 14 hr post-feeding) from day 0 to day 10 and at 0, 4, 8, and 12 hr post-feeding on day 11. All measurements designated as time 0 were taken immediately prior to feeding; this is equivalent to 24 hr after fresh feed was last provided. On days 12 through 14 and on d 17 and d 21, ruminal samples were obtained twice each day (0 and 8 hr post-feeding). Samples were taken 8 hr post-feeding on the remaining days.

Ruminal fluid and blood glucose concentrations were measured twice daily on days 0 through 10 (0 and 8 hr post-feeding) and at 8 hr post-feeding on days 11, 13, 15, 17, 19, and 21. Ruminal and blood osmotic pressures were measured at 0 and 8 hr post-feeding during days 0 through 10. Blood pH, bicarbonate and packed cell volume also were monitored during the trial. These

blood parameters were analyzed from samples obtained at 0 and 8 hr post-feeding on days 0 to 10 and at 8 hr post-feeding on the remaining days. To determine establishment of the inoculated microbes, ruminal fluid was obtained 0 hr post-feeding on days 0, 4, 7, 10, 14, 17, and 21 and plated on selective media at dilutions of 1×10^{-2}, 1×10^{-3}, 1×10^{-4}, and 1×10^{-5}.

Trial 2. Eight yearling heifers were housed two to a pen to instigate greater competition for feed and thereby increase feed intake. These heifers had ad libitum access to their diets and were adapted from a roughage to a concentrate diet in 5 days as outlined in Table 7. Diet compositions are found in Table 8. Four of the eight heifers were inoculated with a 5 strain propionibacteria co-culture (41, 63, 81, 89, and 104) daily. The inoculated heifers received 25 mL daily of a culture media containing 2.5×10^{11} cfu of each strain of *Propionibacterium* via the rumen cannula.

Ruminal pH was monitored at 0 and 8 hr post-feeding on days 0 to 3, 6 times each day (0, 4, 8, 10, 12, and 14 hr post-feeding) on days 4 to 7, and at 0, 4, and 8 hr post-feeding during d 8 and 9. Ruminal VFA and lactate concentrations were analyzed on all samples obtained during days 4 to 7. Ruminal fluid and blood glucose concentrations were measured at 0 hr post-feeding on days 0 to 3 and at 0, 4, and 8 hr post-feeding during days 4 to 9. Osmolalities of ruminal fluid and blood were measured on samples obtained at 8 hr post-feeding on d 0 to 3 and d 8 to 9 and at 0 and 8 hr post-feeding on days 4 to 7. Blood parameters (pH, bicarbonate, and packed cell volume) were analyzed on samples obtained at 0 hr post-feeding on days 0 to 3 and day 8 and at 0 and 8 hr post-feeding on days 4 to 7. Ruminal fluid samples were plated to determine populations of propionibacteria strains on days 0, 3, 5, and 7 at 0 and 4 hr post-feeding. Dilutions for the rumen fluid on the selective agar were 1×10^{-2}, 1×10^{-3}, and 1×10^{-4}.

Trial 3. Fourteen animals (6 steers and 8 heifers) were used in this trial. These cattle were allotted to two groups with 7 cattle in each group. One group was inoculated with the 5 strain propionibacteria co-culture (41, 63, 81, 89, and

104) daily beginning 14 days prior to the onset of feeding the 50% concentrate diet and throughout the trial. These cattle were dosed intraruminally with 25 mL of a culture containing 2.5 X 10^{11} cfu/mL of each of the 5 strains of *Propionibacterium*. These animals were fed a 50% concentrate diet for 5 days after which the diet was abruptly change to 90% concentrate (Table 8). After being fed the 90% concentrate diet for 1 day, feed was withheld for 24 hr. For the next 2 days, each animal was given 2.5% of its body weight of the 90% concentrate diet at 0800. Any animal that did not consume its allotment of feed by 0900 received the remaining feed intra-ruminally via cannula. Thereafter, each animal was provided with feed at the rate of 1.5% of body weight each day.

Ruminal parameters (pH, VFA and lactate concentrations, glucose concentrations, and osmotic pressure) were measured at 0 and 8 hr post-feeding on days 1 to 4 and at 0, 4, 6, 8, 10, and 12 hr post-feeding on d 5. During the day of feed withdrawal (d 7) these parameters were measured only at 0 hr post-feeding. When cattle were fed the 90% diet (d 6 and d 8 to 9), all parameters were monitored 7 times each day (0, 4, 6, 8, 10, 12, and 14 hr post-feeding). Ruminal samples taken at 0 hr post-feeding on day 23 (while inoculum was still being administered) were cultured to determine the establishment of the inoculated propionibacteria strains. The ruminal fluid was plated on selective media at dilutions of 1 X 10^{-3}, 1 X 10^{-4}, and 1 X 10^{-5}.

Laboratory procedures. All ruminal samples were obtained without opening ruminal cannulas by attaching a vacuum pump to an indwelling ruminal tube with a stainless steel filter tip; this tube passed through a hole in the cannula plug. At each sampling, 100 mL of ruminal fluid was collected from each animal. During trials 1 and 2, 10 mL of blood was collected from indwelling jugular catheters and placed into 10 mL vacuum tubes containing sodium heparin. Another 10 mL of blood was collected into a syringe and transported to the laboratory for centrifugation. All vacutainers of blood were placed on ice for transport to the laboratory for blood-gas analysis.

Blood pH and bicarbonate concentrations were analyzed using a blood gas analyzer (model 1304; Instrumentation Laboratory, Lexington, MA). Packed cell volumes were determined using the same sample by drawing whole blood into heparinized micro-hematocrit capillary tubes (W.H. Curtin and Co.) and sealed with Critoseal ® clay. These tubes were centrifuged for 5 minutes in a capillary centrifuge (Model L411 Phillips-Drucker, OR) and interface heights were measured (International Micro-Capillary Reader, International Equipment Co., Needham Hts., MA).

The 10 mL of blood collected in the syringe was dispersed into microcentrifuge tubes and centrifuged at 5500 X g for 10 min. Blood plasma was decanted into new tubes that were frozen and analyzed later for glucose concentration and osmolality.

The 100 mL of rumen fluid collected was divided into 50 mL aliquots. One aliquot was placed on ice and transferred immediately to the laboratory for microbial plating. The other aliquot was used for analysis of all other ruminal parameters. To check ruminal pH values, a small aliquot was poured into a 10 mL beaker and pH was measured (Digi-Sense ® LCD portable pH meter, model 5994-10; Cole Parmer Instrument Co., Chicago, IL). The remaining sample was spun in microcentrifuge tubes at 5500 X g for 10 min. and diluted; the supernatant fluid was placed in another tube for transport to the laboratory for VFA and lactate analysis. In preparation for HPLC analysis, 0.5 mL of this supernatant was placed into 0.5 mL of 10 mM sulfuric acid. This mixture was filtered through 0.2 um filters directly into 2 mL HPLC autosampler vials. These samples were injected into a the HPLC (Hewlett Packard HPLC system equipped with a diode-array detector; Hewlett Packard, Atlanta, GA). Concentrations of lactic acid and VFA were calculated based on retention times and peak heights of known external standards measured at 210 nm.

For glucose analysis, prepared plasma and ruminal fluid samples were assayed (Rapid Stat ™ Kit and Glucose Rapid Stat Reagent, Pierce Chemical Co., Rockford, IL). Osmolality of plasma and rumen fluid was analyzed by

freezing point depression (Osmette A - Automatic osmometer model 5002; Precision Systems, Inc., Natick, MA).

Statistical Analysis. In trial 1, all measurements were analyzed using a 2 x 2 factorial split-split plot design. The data from trials 2 and 3 were analyzed using a split plot design. Treatment differences were evaluated using a t-test (Steel and Torrie, 1980).

Results and Discussion

Trial 1. Daily DM intake by treatment during the adaptation periods are shown in Table 9. Maximum DM intake allowed on any day was 13.6 kg. Mean intakes were not different ($P>0.05$) either due to inoculation or among adaptation periods. Daily intakes fluctuated among days. Surprisingly, DM intake by steers tended to decrease earlier for steers on the 9 day adaptation scheme than for steers on the 5 day adaptation scheme.

There were no significant differences ($P>0.05$) in ruminal pH with treatment or adaptation period. Ruminal pH did not decline to the point of acidosis during either adaptation period. However, during the remainder of the trial, pH declines indicated that acidosis occurred in every steer except for one that had been inoculated. Of the seven steers that exhibited acid pH values for ruminal contents, two steers maintained acid ruminal pH values (below 5.2) for several days, one being a control animal on the 9 d adaptation scheme and the other being an inoculated steer on the 5 d adaptation scheme.

Ruminal pH was altered ($P<0.01$) by sampling day and time within day; means for the total trial are illustrated in Figure 3. Mean ruminal pH reached its lowest point on days 14 and 18. During the first 10 d, ruminal pH for the steers decreased linearly; this was associated with an increase in concentrate content of the diet. Thereafter, pH fluctuated. Within the day, mean ruminal pH was lower ($P<0.01$) at 10 hr post-feeding than at 0 or 4 hr post-feeding (5.88 vs. 6.05 and 6.06).

Neither ruminal lactate nor VFA concentrations were altered significantly ($P>0.05$) by inoculation. During the adaptation period, lactic acid content of

ruminal samples never exceeded 3.0 mM. Even during the remainder of the trial when low ruminal pH reflected acidosis, lactic acid remained quite low. Of the seven steers that exhibited acidic pH values, only three had detectable lactate in ruminal samples, the highest being 20.87 mM and the mean for these three steers being 8.71 mM. These low values are not consistent with other more acute acidosis studies that routinely observe lactate concentrations exceeding 50 mM when ruminal contents are acidic (Dunlop, 1972; Uhart and Carroll, 1967).

Ruminal glucose concentrations were not significantly ($P>0.05$) altered by inoculation nor different among adaptation periods, but a difference due to sampling date was detected ($P<0.01$) as shown in Table 10. Glucose concentrations increased with days on feed being lowest on day 1 at 0.08 mM and the highest on day 21 at 4.85 mM. This represents a 60-fold increase during the trial. Ryan (1964) also detected large increases in ruminal glucose concentrations following the addition of wheat to the rumens of sheep.

Ruminal osmolalities were not affected ($P>0.05$) by either inoculation or adaptation time although again day-to-day differences were apparent ($P<0.01$). Mean osmotic pressures of ruminal samples during the first 10 days on trial (Table 11) show no consistent time pattern ($r^2=0.51$). Osmolality averages were considerably higher than the 275 to 300 mOsm means noted for heifers limit fed high concentrate diets reported by Garza et al. (1989) and were quite variable from day to day.

Blood measurements (pH, glucose concentration, and osmotic pressure) were not altered by inoculation during either adaptation period. This is not surprising because acidosis was not encountered during the adaptation period. However, an inoculation by adaptation period interaction was detected ($P<0.05$) for blood bicarbonate levels. For steers on the 9 d adaptation scheme, control steers maintained a higher blood bicarbonate concentration than inoculated steers (29.1 mM vs. 27.8 mM) whereas with the 5 d adaptation scheme, bicarbonate concentrations were higher for inoculated steers (29.4 vs. 27.9 mM).

Differences in packed cell volume due to inoculation or adaptation time were not significant ($P>0.05$) although, again, differences were detected among days ($P<0.01$); packed cell volume tending to decline with adaptation to the higher concentrate diet (Table 12). Counts of *Propionibacterium* strains in ruminal fluid yielded values that ranged from 0 to 1.1 X 10^5 indicating that the strains inoculated were present at lower concentrations than expected from inoculation alone. There were only 7 detected colonized plates out of 112 plates.

Trial 2. Mean DM intake for control and inoculated heifers in trial 2 are presented in Table 13. Inoculation did not alter ($P>0.05$) DM intake. However, on the second day that the 90% diet was fed (day 6), DM intake dropped noticeably for both inoculated and control heifers and remained low for the remainder of the trial. Mean daily DM intake dropped from 23 kg on d 4 to 18 kg on d 9. Such decreases in intake are expected when cattle are being adapted to concentrate diets (Slyter, 1976; Leedle et al., 1995). Nevertheless, on day 9 the mean NEg intake for the heifers was greater (10.8 vs 8.9 Mcal). Consequently, energy intake had increased despite the decrease in dry matter intake.

Inoculation had no significant ($P>0.05$) effect on ruminal pH or ruminal concentrations of VFA, lactate, or glucose. From day 0 to day 9, ruminal pH of control heifers dropped from 7.02 to 5.56 while ruminal pH of inoculated heifers dropped from 6.93 to 5.65; these are changes of 1.46 and 1.29 pH units for control and inoculated heifers, respectively. Neither difference or this change was significantly ($P>0.05$) different. The change in hydrogen ion concentration for the control heifers was 2.7 X 10^6 while the change in those inoculated was 2.1 X 10^6; this difference ($P>0.05$) is 6.0 X 10^5. Each heifer in this trial exhibited a ruminal pH indicative of acidosis at some time during the trial. However, ruminal lactic acid accumulated in only one case, with a non-inoculated heifer on day 6 when lactate concentration reached 19 mM when ruminal pH value was 4.68. These values are not consistent with values reported by Briggs et al.

(1957) who found that lactic acid concentration in the rumen of sheep exceeded 20 mM when ruminal pH values were below 5.0 .

Ruminal osmotic pressures were not altered ($P>0.05$) by inoculation with propionibacteria, but again a day effect was detected ($P<0.05$) as shown in Table 14. Osmolality was highest on day 6 of the study at 385 mOsm/kg.

Blood parameters (pH, packed cell volume, osmotic pressures, glucose and bicarbonate concentrations) were not significantly altered ($P>0.05$) by inoculation. However, blood bicarbonate was lower ($P<0.05$) when cattle were fed the higher concentrate diets (75 and 90%) than when fed the lower concentrate levels (30, 45, and 60%). Selective plating of *Propionibacterium* yielded counts between 0 and 2.2×10^5, none of which were above the expected from inoculation alone (18 colonized plates out of 72 total plates).

Trial 3. Ruminal pH values during this study was not affected ($P>0.05$) by inoculation. When the 90% concentrate diet was being fed, each animal had a ruminal pH value below 5.3 indicative of acidosis. All but one calf had very acid ruminal pH values on the second day that cattle were engorged with the 90% diet (d 9). During the days of engorgement two treated animals that did not need to be forced-fed on either day. These animals consumed the ration on their own. On day 9, the second day of grain engorgement, mean ruminal pH was 5.44 for control cattle vs. 5.20 for inoculated cattle. This suggests that the propionibacteria co-culture was not preventing the pH drop characteristic of acute acidosis. However again, despite the low pH, ruminal lactate did not accumulate above a concentration of 20 mM. Inoculation did not ($P>0.05$) alter ruminal osmolality, VFA, lactate, or glucose concentrations.

No evidence of propionibacteria establishment within the rumen was detected. Together with the previous study, this leads one to question why inoculation was not preventing acidosis. Perhaps microbial interactions between inoculated and inherent ruminal strains or interactions among the strains of *Propionibacterium* being introduced to the rumen are involved. Based on in vitro studies by Davidson (1997), some bacteriocin activity existed with the strains

used in these co-cultures. Such an interaction could hinder microbial growth and thereby reduce the antacid properties of these strains of propionibacteria. Based on this concern, subsequent studies were conducted in vitro. Based on those studies and a concern about interactions among strains, propionibacteria strain 63 alone selected as being optimum for increasing lactic acid use and survival at an acidic pH (Parrott, 1996; Davidson, 1997) was chosen to use in subsequent studies.

Selective microbial plating using lactic acid as a substrate indicated that certain animals, even though they had not been inoculated, had substantial populations of native lactate utilizers in the rumen. These observations may explain why it proved difficult to produce acute lactic acidosis in some of these cattle.

Implications

The co-cultures of *Propionibacterium* used in these trials as a ruminal inoculum failed to prevent drops in ruminal pH to values below 5.0. Recovery of inoculated propionibacteria strains was very low. Whether interactions between the specific strains or other factors reduced establishment is not known. Yet, based on ineffectiveness of these co-cultures, further studies are needed using a single strain of propionibacteria as an inoculum to prevent acidosis.

Table 7. Adaptation schemes for trial 1 and trial 2.

Day	5 Day Period	9 Day Period
	% Concentrate	
1	30	30
2	45	30
3	60	45
4	75	45
5	90	60
6	90	60
7	90	75
8	90	75
9	90	90
10-21	90	90

Table 8. Composition of diets (DM basis) for trials 1, 2, and 3.

Feed Ingredients (%)	30%	45%	50%	60%	75%	90%
Ground corn	22.1	36.9	42.2	51.8	66.9	82.0
Cottonseed Hulls	35.1	26.2	20.8	17.7	7.6	0.0
Alfalfa Pellets	35.1	29.2	29.2	22.7	17.7	10.2
B-075 supplement[a]	7.7	7.7	7.8	7.8	7.8	7.8

[a] composition of the supplement are presented in table 2 of chapter III.

Table 9. Mean DM intakes for the steers in trial 1 during the adaptation periods (kg/d).

Day	5 Day Adaptation		9 Day Adaptation	
	Control	Treated	Control	Treated
1	13.6	13.6	13.6	13.6
2	13.6	13.6	13.0	13.6
3	13.6	13.6	10.9	12.5
4	10.1	10.0	12.7	12.4
5	10.2	13.4	10.9	12.3
6	11.8	11.1	13.5	13.4
7	10.6	09.3	12.7	13.6
8	09.8	07.7	04.8	10.9
9	10.5	11.8	11.5	09.3

Figure 3. Ruminal mean pH values for all animals in trial 1.

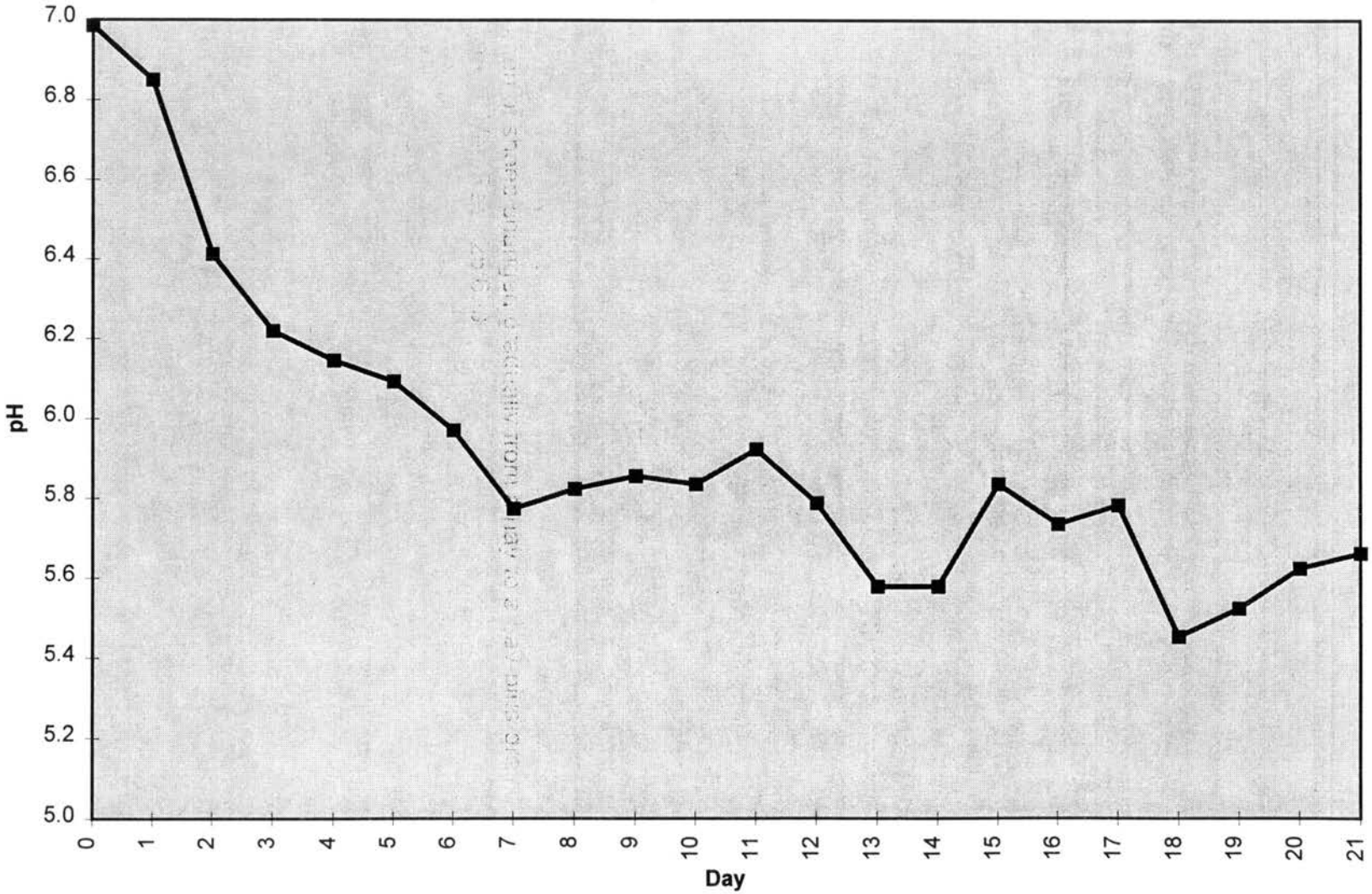

Table 10. Daily mean ruminal glucose concentrations for all steers during trial 1.

Day	Glucose concentrations (mM)
1	0.08^{a}
2	0.18^{a}
3	0.33^{a}
4	0.98^{ab}
5	1.54^{b}
6	1.92^{b}
7	2.14^{bc}
8	2.89^{bc}
9	3.85^{d}
10	3.11^{d}
11	3.08^{d}
13	3.43^{d}
15	3.13^{cd}
17	2.00^{bc}
19	3.65^{d}
21	4.85^{d}

^{a,b,c,d}Means within a row lacking a common superscript differ (P<0.01).

Table 11. Mean rumen osmolalities for all steers during the first 10 days of trial 1.

Day	Osmotic Pressure (mOsm/kg)
1	284^{ab}
2	307^{abc}
3	277^{ab}
4	313^{abc}
5	326^{bc}
6	317^{bc}
7	352^{d}
8	335^{bcd}
9	302^{abc}
10	361^{d}

^{a,b,c,d}Means within a row lacking a common superscript differ (P<0.01).

Table 12. Daily mean blood packed cell volume for all steers during the first 10 days of trial 1.

Day	Packed cell volume, % of total volume
1	31.0[a]
2	30.0[a]
3	29.3[ab]
4	30.1[a]
5	29.3[ab]
6	27.5[b]
7	27.2[b]
8	27.6[b]
9	27.2[b]
10	27.5[b]

[a,b]Means within a row lacking a common superscript differ (P<0.01).

Table 13. Daily DM intakes for heifers during trial 2 (kg/d).

Day	Control	Treated
0	27.3	27.3
1	27.3	27.3
2	31.8	31.8
3	19.3	21.0
4	27.0	19.7
5	23.6	22.5
6	11.5	13.3
7	15.9	14.5
8	17.7	18.7
9	19.8	16.1

Table 14. Mean daily ruminal osmotic pressures for all heifers during trial 2.

Day	Osmotic Pressure (mOsm/kg)
0	254[ab]
1	272[abc]
2	303[bc]
3	297[abc]
4	328[c]
5	338[c]
6	385[d]
7	301[bc]
8	328[c]
9	298[abc]

[a,b,c,d]Means within a row lacking a common superscript differ (P<0.05).

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CHAPTER V.

TEMPORAL EFFECTS OF ACUTE ACIDOSIS IN CATTLE

Abstract

The time course of ruminal and blood changes associated with acute acidosis was studied using three forage-fed steers (455 kg). To induce acidosis, these steers were ruminally dosed with a starch-slurry providing starch at 1.25% of body weight. Because ruminal pH had not dropped 4 hr after dosing, each steer was offered 9 kg of finely ground corn; corn was fully consumed within one hour. By 12 hr post-engorgement, approximately 7 hr post-feeding, ruminal pH had declined from 7.4 to 4.8. The first noticeable decline in blood pH was 2 hours later when blood pH had decreased to 7.32, an index of systemic acidosis. Ruminal lactate concentrations rose from below 1 mM to 136 and 162 mM at 12 and 14 hr post-engorgement. Glucose accumulation in the rumen preceded lactate accumulation reaching a maximum concentration of 21.6 mM; increases in blood glucose also were evident. By 14 hr after engorgement, alkali reserves of blood were depleted; this permitted blood pH to fall. Both ruminal and blood osmolality increased slightly during acidosis. When devising ideal microbial inoculants to prevent acidosis, researchers must consider not only ruminal lactate but also accumulation of free glucose in the rumen that precedes lactate accumulation. In the absence of ruminal samples, measurement of base excess in peripheral blood may help to detect early stages of acidosis, to assess the severity of the condition, and to appraise cause of death.

(Key Words: Acidosis, Acute acidosis, Cattle, Blood, Rumen)

Introduction

Acidosis in ruminants is defined as a condition involving high ruminal acid concentrations accompanied by pathological blood acidity. Based on severity, ruminal acidosis generally is divided into two classes: acute and chronic

(subacute). According to Horn et al. (1979) and Britton (1985), acute acidosis poses a life-threatening situation as a result of physiological alterations whereas chronic acidosis is associated with economic losses as a result of decreased animal performance (i.e., reduced feed intake and weight gain).

Acidosis can be induced experimentally by manually dosing large amounts of rapidly fermented substances into the rumen. Specific ruminal and blood changes associated with manual grain engorgement have been reviewed by Slyter (1976). The object of this experiment was to examine time course of the change in ruminal and blood measurements in cattle with induced acidosis.

Materials and Methods

Three ruminally cannulated steers averaging 455 kg body weight housed in metabolism stalls were used in this study. They had been maintained for several months previously on a diet of native pasture and had ad lib access to prairie grass hay plus 1 kg daily of a soybean meal-based protein supplement. This experimental design was approved by the Animal Care and Use Committee. To initiate acidosis, feed was withheld for 24 hr after which corn starch, mixed with water to form a slurry, was dosed into the rumen via cannula at a rate of 12.5 g of starch per kg body weight following the procedures of Nagaraja et al. (1981) and Nagaraja et al. (1985). When no decline in pH was detected 4 h after dosing, each steers was offered 9 kg of finely ground corn grain. All corn was consumed by each animal within 60 min.

Ruminal and blood parameters were monitored 2 hr prior starch engorgement and at 2, 4, 6, 8, 12, and 14 hr after starch was dosed. Ruminal fluid samples were taken using a vacuum pump connected to tubes dwelling in the rumen and equipped with stainless steel filters. Measurements on ruminal fluid included pH, osmolality, and concentrations of glucose, VFA and lactate. Blood, drawn by tail venipuncture, was assayed for pH, partial pressure of carbon dioxide and oxygen (pCO_2 and pO_2), bicarbonate concentration (HCO_3^-), base excess, hematocrit, osmolality, and glucose concentration.

Ruminal pH was determined using a small aliquot of rumen fluid with a combination electrode and a pH meter (Accumet ® model 1003 portable pH meter, Fisher Scientific, Pittsburgh, PA). The remaining ruminal fluid was centrifuged at 5500 X g for 10 min. The supernatant fluid was decanted and frozen (-20^{O} C) for later analysis. Later, these samples were assayed for concentrations of VFA, lactate, and glucose and osmotic pressure. Blood was collected into 3 mL Na^{+}-heparinized vacuum tubes for blood gas analysis and into a 10 mL EDTA vacuum tube for glucose and osmolality measurement. All 3 mL tubes of blood to be transported for pH and blood gas analysis were placed on ice immediately. Blood collected in 10 mL tubes was immediately centrifuged at 5500 X g for 10 min. Plasma was drawn off, placed into microcentrifuge tubes, and frozen for later analysis.

Samples of the supernatant fluid from ruminal contents to be assayed for VFA and lactate were defrosted at a slow rate and prepared for HPLC analysis (Hewlett Packard 1090 High Pressure Liquid Chromatography system equipped with a diode-array detector, Hewlett Packard, Atlanta, GA). The supernatant fluid (0.5 mL) was transferred to a second tube and acidified with an equal volume (0.5 mL) of 0.01 M sulfuric acid solution. This mixture was filtered through a 0.2 um filter into 2 mL HPLC autosampler vials. These samples were injected into 0.005 M H_2SO_4 mobile phase heated to 65^{0} C and separated using a Bio-Rad HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA); peaks detected at 210 nm were compared to known external standards.

Glucose concentrations of both ruminal fluid and blood were determined using a procedure adapted from the Rapid Stat ™ Kit and the Glucose Rapid Stat Reagent (Pierce Chemical Co., Rockford, IL) that uses a single reagent procedure for the quantitative colorimetric determination of glucose. Intensity of the blue-green color was measured spectrophotometrically (Gilford Response UV-VIS spectrophotometer, Gilford Instrumentation, Oberlin, OH) at 630 nm and compared against glucose standards.

Blood pH and gas analyses were performed using a 288 Ciba Corning Blood Gas Analyzer. This blood gas analyzer measured pH, pCO_2, pO_2, HCO_3^-, base excess, and hematocrit. Osmotic pressures of blood and ruminal fluid were measured by freezing point depression using an osmometer (5002 Osmette Automatic Osmometer, Precision Systems, Inc., Natick, MA).

The steers used in this study were treated for acidosis by introducing sodium bicarbonate into the rumen via the cannula. Rumens also were evacuated. Fluid from a roughage-fed steer was then placed into the rumen to re-inoculate and stabilize the rumen. After treatment steers were placed on their previous diet and watched. All steers recovered from the acidotic conditions.

Results and Discussion

For the first 8 hours, ruminal pH persistently remained above 7.0 (Table 15) averaging 7.73. However, by 12 hr post-engorgement and approximately 7 hr after the corn grain was consumed, ruminal pH had declined drastically to a mean of 4.77. Even at this time, blood pH remained at 7.40, very close to the -2 hr value of 7.42. However, by 14 hr post-engorgement, blood pH had declined to 7.32 (Figure 4), a condition classified as systemic acidosis. Vestweber et al. (1974) measured blood pH of sheep at 7.44 and 7.36 before and after producing acute acidosis in sheep. By 14 hr after engorgement, ruminal pH had declined to 4.47 for a total drop of 3.36 pH units.

Table 16 presents blood parameters for the 3 steers. pCO_2 tended to decrease with time for the first 6 hours post-engorgement. However, at 14 hr post-engorgement, when rumen and blood pH had decreased to 4.47 and 7.32, respectively, pCO_2 had increased to 50.73 mm Hg, a 25% increase in two hours. Howard (1981) concluded that because lactate is oxidized to CO_2 by the liver and kidneys, this should increase pCO_2. An increased pCO_2 also has could be attributed to an increased intensity of ruminal fermentation and microbial CO_2 production (Leedle et al., 1995). pO_2 concentration in the blood fluctuated over time but decreased markedly 14 hr post-engorgement in concert with the rise in pCO_2. Because extra CO_2 is produced to buffer blood lactic acid, venous blood

pO_2 drops as more O_2 diffuses from blood to tissues. The fact that this is venous blood is important because venous blood contains CO_2 being carried from the tissues to the lungs.

Blood HCO_3^- remained relatively steady at approximately 30.4 mM for the first 8 hr post-engorgement. However, by 12 hr post-engorgement, HCO_3^- had declined by 5.6 mM to 24.8 mM when ruminal pH had declined 4.77 and blood pH had begun to decline. Base excess ranged from 5.57 to 7.80 mM (mean of 6.64 mM) during the first 8 hr post-engorgement. But by 12 hr post-engorgement, base excess had exhibited a sharp decline to 0.75 mM, a difference of -5.89 mM; the drop continued to so that at 14 hr post-engorgement, mean base excess was negative (-0.63 mM) due primarily to decreased HCO_3^-. Similar decreases in base excess during acidosis were reported by Nagaraja et al. (1982).

Hematocrit, calculated from blood gas analysis, remained fairly stable until ruminal pH declined at 12 and 14 hr post-engorgement when hematocrit reached 37.2 and 38.7 compared with a mean of 32.4 earlier. Nagaraja et al. (1982) reported that hemoconcentration was characteristic of acidosis based on slight increases that they observed in packed cell volume of blood.

During the onset of ruminal acidosis, acetate and lactate concentrations changed markedly (Table 17). Acetate concentration at 8 hr post-engorgement spiked to 26.6 mM but then declined as lactate concentration increased to 136 and 162 mM at 12 and 14 hr post-engorgement, respectively (Table 5 and Figure 5). These high ruminal lactate concentrations are consistent observations by Dunlop (1972) who observed lactate concentration of 150 mM when ruminal pH had dropped to 4.2. During this time, acetate concentration also declined; at 14 hr post-engorgement. Nagaraja et al. (1982) noted that ruminal lactate concentration increased dramatically and total ruminal VFA declined sharply in steers made acidotic by intraruminally dosed glucose. These lactate concentrations far exceeded the levels found in our previous trials (maximum of

about 35 mM) in which ruminal pH had been measured with the more chronic acidosis during adaptation to a high concentrate diet.

Ruminal and blood glucose concentrations are presented in Table 18. Normally, ruminal glucose concentrations are extremely low as noted during the first 6 hr post-engorgement when concentration averaged 0.22 mM. However, at 8 hr post-engorgement, ruminal glucose concentrations increased 20-fold to a value of 4.6 mM. Increases continued the next 6 hr so that by 14 hr post-engorgement, glucose concentrations had increased by nearly 100-fold. Ryan (1964) found unusually high ruminal glucose concentrations (13.5 mM) in sheep following the addition of large amounts of wheat to the rumen. He suggested that this high concentration of glucose preceded the drop in ruminal pH as noted in our trial.

Two explanations for these increases in glucose concentration have been advanced. First, utilization of glucose by ruminal microbes might be inhibited. The ability of protozoa to engulf small starch particles and attenuate fermentation might have been exceeded. The spike in acetate concentration at this time might reflect such a change in bacterial or protozoa metabolism. Slyter (1976) indicated that when ruminal glucose concentrations is higher, metabolism of lactic acid to acetic and propionic acids is inhibited. Thereby, high ruminal glucose concentrations may cause lactate to accumulate. Interest in ruminal glucose has been prompted from such studies. The correlation between ruminal lactate and glucose concentrations during acute acidosis was quite strong ($r = 0.98$) in this study (Figure 6).

An alternative explanation for glucose accumulation is that glucose production rate is increased. Microbial amylase released from lysing microbes, particularly protozoa, could accelerate hydrolysis of soluble starch to glucose as proposed by Slyter (1976).

Blood glucose concentrations remained steady during the first 6 hr post-engorgement (mean of 3.74 mM). During the next 8 hr, accompanying ruminal glucose accumulation, blood glucose rose so that by 14 hr post-engorgement,

blood glucose concentration was 34% above the earlier mean. Leedle et al. (1995) also noted that glucose concentrations of both blood and ruminal contents increased when a 90% concentrate diet was fed to cows. Whether this increase is due to an increased glucose supply (from hepatic synthesis from VFA, glycogenolysis or absorption) or decreased utilization is not known.

Rumen osmolalities increased gradually with time from 2 hr to 14 hr post-engorgement. The mean osmolality at 2 h post-engorgement was 238 mOsm/kg; during acidosis it was 289 mOsm/kg. This is a difference of 51 mOsm/kg. No abrupt change in osmolality preceded glucose accumulation in the rumen that would osmotically shock protozoa to cause release of amylase. Therefore, if protozoal lysis is involved with ruminal glucose accumulation, some factor other than osmolality must be responsible for such lysis.

Eadie and Mann (1970) proposed that damage to the ruminal wall during acidosis could be attributed to high ruminal osmolality and the ensuing rapid movement from plasma into the rumen causing sheets of mucosa to be stripped from the rumen wall. Ahrens (1967) reported that ruminal osmolality that exceeded 500 mOsm/kg in heifers with acidosis. Changes we noted were much smaller than those. However, because plasma fluid moves into the rumen to counterbalance osmolality, changes in ruminal fluid osmolality alone may not fully detect the impact of acidosis on fluid movement through the ruminal wall.

Blood osmotic pressures remained fairly constant with a mean osmolality of 296 mOsm/kg. Tonicity of blood normally is maintained at approximately 300 mOsm/kg (Carter and Grovum, 1990). During acidosis, Huber (1971) detected that tonicity of ruminal contents relative to plasma changed from being hypotonic to being hypertonic. He concluded that this is an important factor in the physiopathology of lactic acidosis. In this the difference between ruminal and blood osmolality decreased over time with isotonicity being reached by 14 hr post-engorgement. This suggests that osmotic damage to the ruminal wall should have been minimal during this time period.

Implications

Acute acidosis was induced by starch engorgement combined with feeding of finely ground corn grain. During induced acute acidosis, ruminal pH fell and lactic acid accumulated to high levels (> 162 mM) some 6 to 8 hr after engorgement. In previous experiments with acidosis during diet adaptation, lactic acid accumulation was less extreme (maximum of 20 mM). Consequently, ruminal inoculation with microbes to use lactic acid, though helpful to prevent acute acidosis, may not reduce the incidence of chronic acidosis. Ruminal accumulation of glucose preceded lactic acid accumulation. Increased activity of ruminal microbes that catabolize glucose may help to reduce the incidence of either chronic or acute acidosis.

Table 15. Mean rumen and blood pH values during acute acidosis.

Hour[a]	Rumen	Blood
	pH	
-2	7.83	7.42
+2	7.93	7.45
4	7.83	7.47
6	7.70	7.46
8	7.40	7.45
12	4.77	7.40
14	4.47	7.32

[a] Hours presented as time post-engorgement.

Figure 4. Mean rumen and blood pH values during acute acidosis.

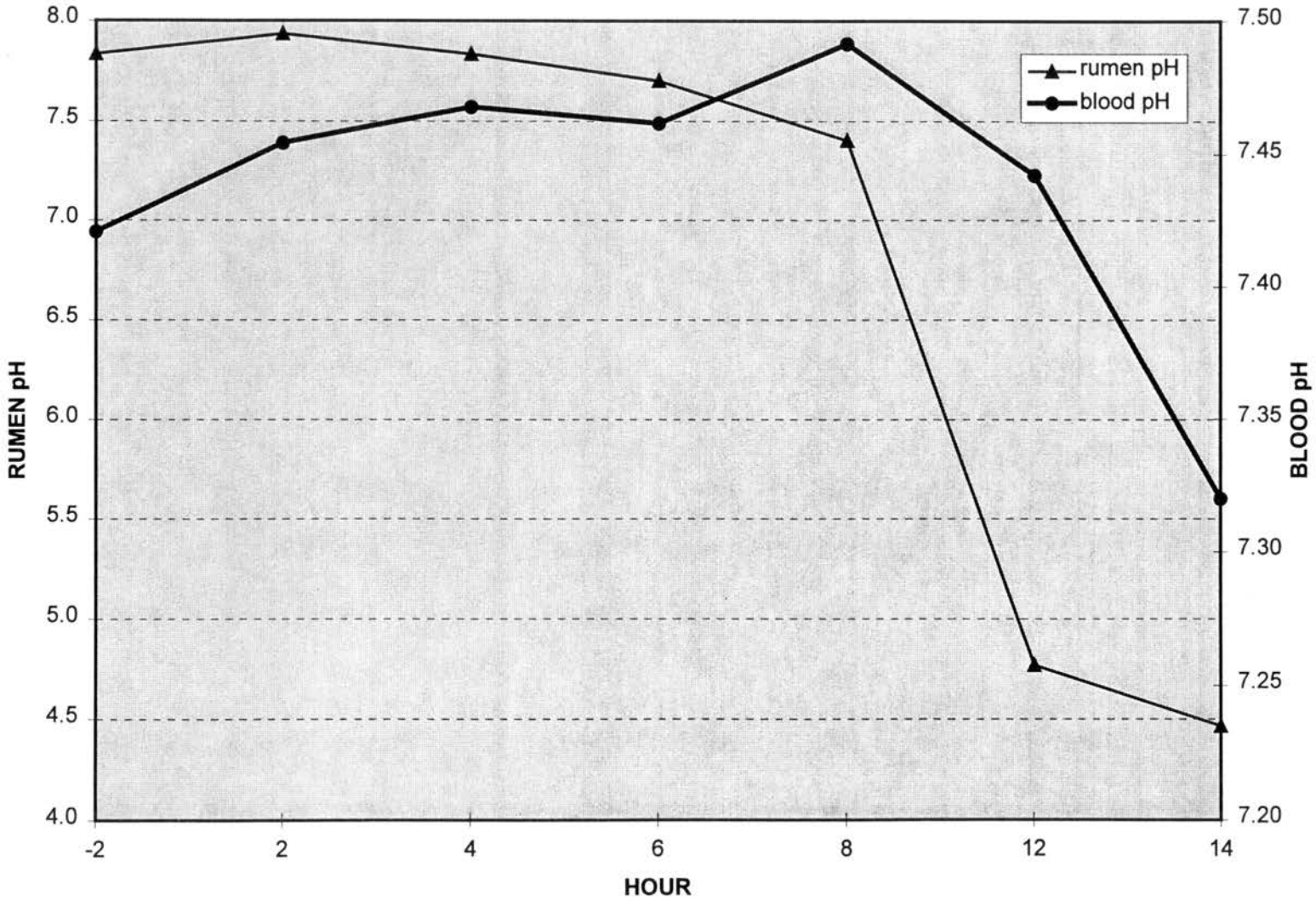

Table 16. Mean blood parameters during the acute acidosis trial.

Hour [a]	pH	pCO_2	pO_2	HCO_3^-	Base excess[b]	Hematocrit
		mm Hg			mM	
-2	7.42	46.83	65.17	30.10	0.00	34.03
2	7.45	44.20	77.37	30.53	0.43	30.47
4	7.47	43.53	72.43	31.30	1.20	31.77
6	7.46	41.40	58.73	29.30	-0.80	34.13
8	7.45	44.05	69.05	30.75	0.65	31.75
12	7.40	40.70	69.85	24.85	-5.25	37.20
14	7.32	50.73	34.20	25.70	-4.40	38.73

[a] Hours presented as time post-engorgement.
[b] Normal HCO_3^- concentration for these steers were 30.10 mM; base excess is the change over time from this baseline value of 30.10 mM .

Table 17. Mean rumen acetate and lactate concentrations during acute acidosis (mM/L).

Hour [a]	Acetate	Lactate
-2	7.70	0.00
2	3.71	0.00
4	3.64	0.00
6	5.59	0.00
8	26.62	0.00
12	4.47	136.47
14	0.00	162.08

[a] Hours presented as time post-engorgement.

Figure 5. Mean ruminal pH compared to mean ruminal lactate concentration.

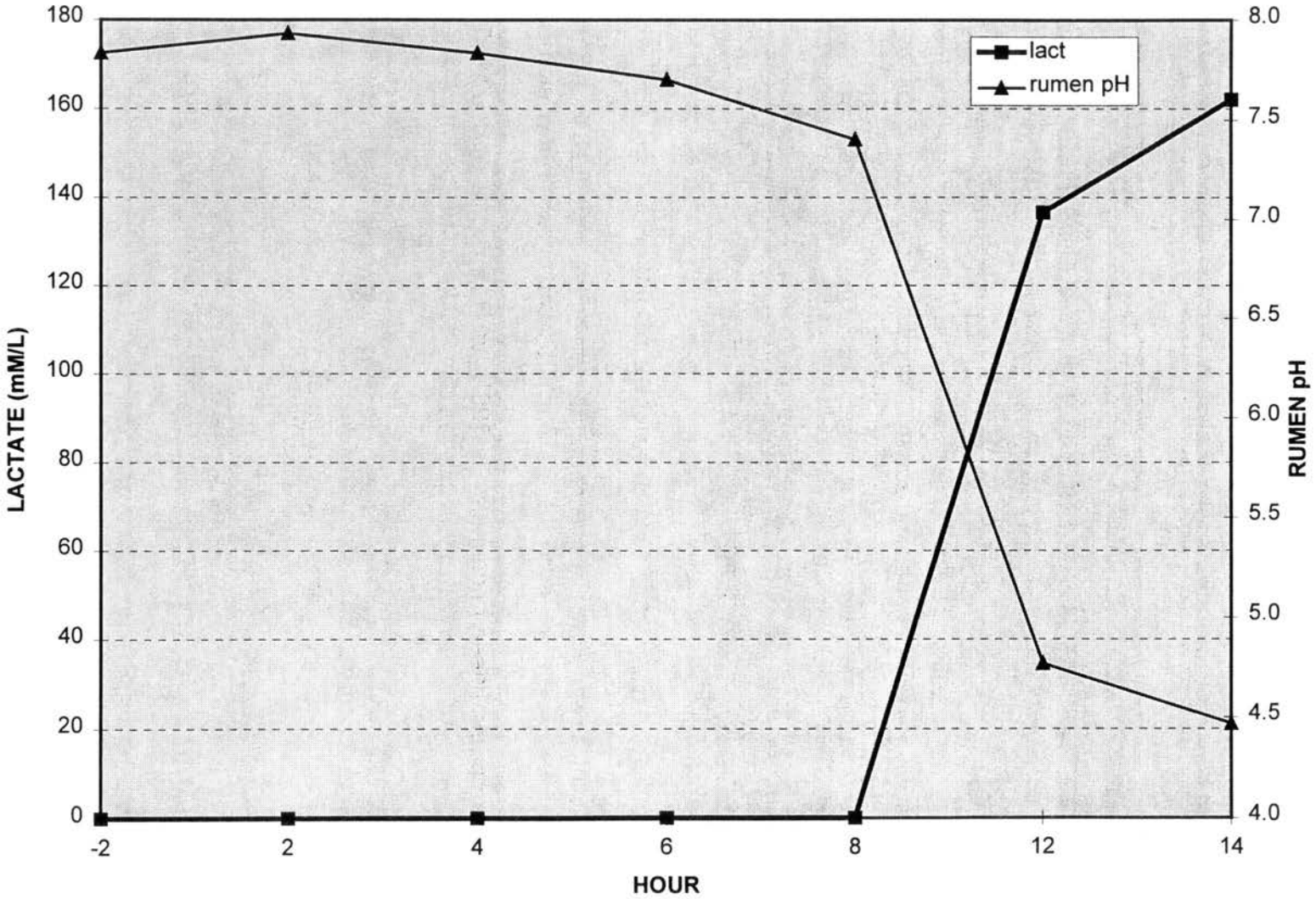

Table 18. Mean rumen and blood glucose concentrations for steers during acute acidosis (uM/mL).

Hour[a]	Rumen [Glucose]	Blood
-2	0.09	3.32
2	0.06	3.95
4	0.05	3.87
6	0.70	3.82
8	4.59	4.53
12	13.44	4.88
14	21.71	5.03

[a] Hours presented as time post-engorgement.

Figure 6. Ruminal lactate concentration compared to ruminal glucose concentration.

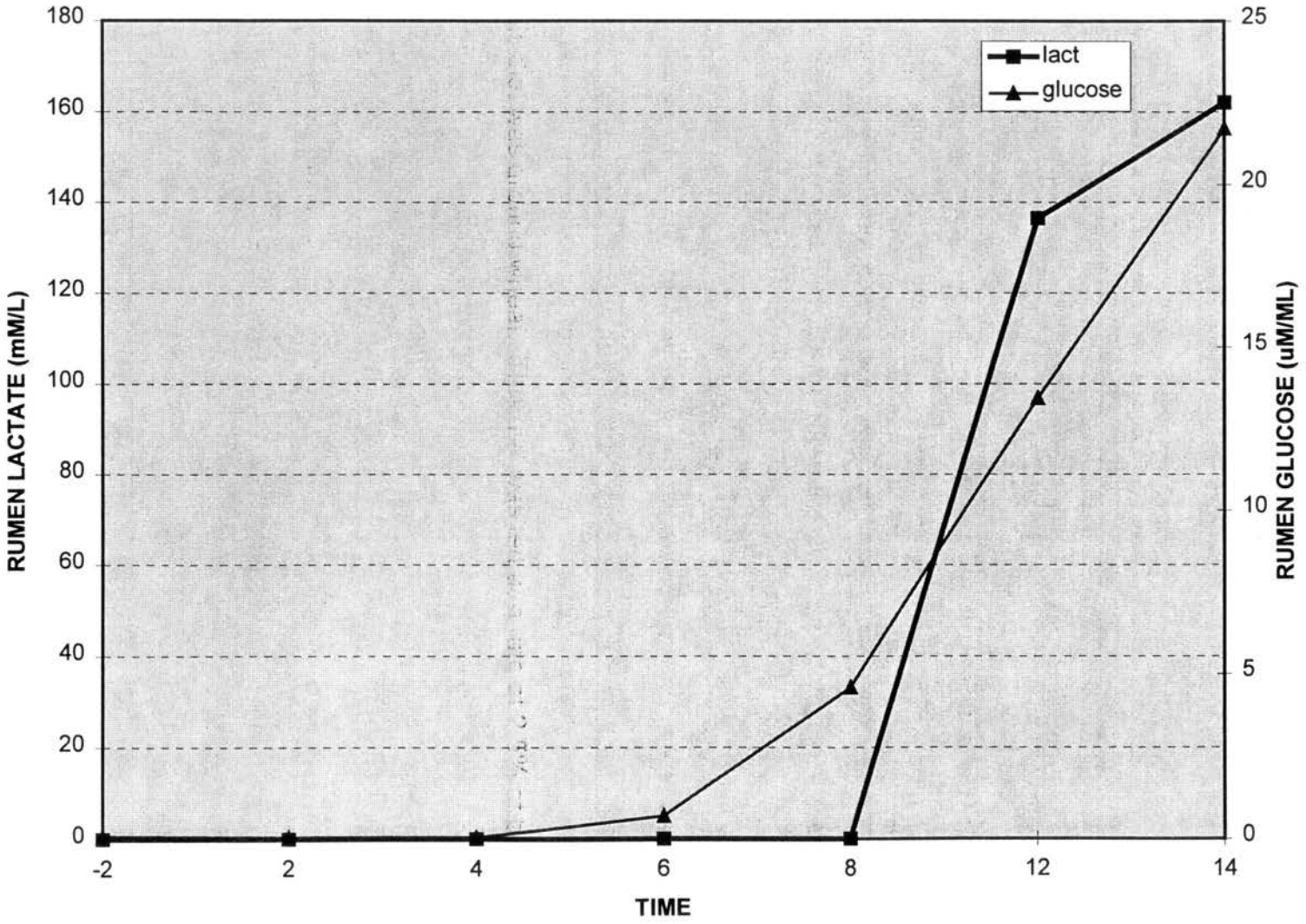

Table 19. Mean ruminal and blood osmolalities for steers during acute acidosis (mOsm/kg).

Hour [a]	Rumen	Blood	Difference
	Osmotic Pressures		
-2	264	287	+23
2	238	291	+57
4	253	294	+41
6	262	300	+38
8	267	298	+31
12	277	301	+24
14	300	300	0

[a] Hours presented as time post-engorgement.

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CHAPTER VI.

IMPACT OF PROPIONIBACTERIA STRAIN P-63 ON ACUTE ACIDOSIS OF FEEDLOT CATTLE

Abstract

This experiment was designed to determine the usefulness of propionibacteria strain P-63 to prevent the sharp decline in ruminal pH and the accumulation of lactic acid during fasting-induced acidosis. Ten ruminally-cannulated heifers were used, five of which received $3x10^{11}$ colony forming units of propionibacteria strain P-63 dosed intra-ruminally each day starting 14 d prior to the introduction of concentrates in the diet. For 5 days prior to the diet switch to a 90% concentrate diet, each heifer was fed a 50% corn-based concentrate diet at 1.5% of body weight each day. On the sixth day, animals were fasted. For the next two days, heifers were offered a diet composed of 90% concentrate. Because wheat diets are notorious for inducing acidosis, 75% of the concentrate portion of this diet was ground wheat. This diet was fed at a rate of 2.5% of body weight each day. Ruminal lactic acid concentration remained lower in inoculated than in non-inoculated heifers. Ruminal pH on day 8 of the trial (the second day on the 90% diet), ruminal pH was greater for inoculated than non-inoculated heifers. Whenever lactic acid accumulated in the rumen, ruminal glucose concentrations had risen. Accumulation of both glucose and lactate in the rumen caused osmotic pressures to increase during acidosis. Data from this experiment were analyzed further following removal of two non-inoculated heifers and one inoculated heifer from the data set based on the fact that ruminal samples taken from the two non-inoculated heifers that showed a substantial number of lactate utilizing bacteria and the one inoculated heifer showed no

establishment of propionibacteria strain P-63. Following this alteration, the difference between inoculated and non-inoculated heifers is even more extreme with lactic acid concentration being higher ($P<0.01$) in non-inoculated heifers during 10, 12, 14, and 16 hr post-feeding on day 7 of the trial and a strong trend for ruminal pH to be lower for non-inoculated heifers during both days 7 and 8. Both lactate and glucose accumulated in the rumen of non-inoculated heifers during day 7 but glucose concentrations were lower in the rumen of inoculated heifers. Osmotic pressures in the rumen during day 7 of the trial were higher ($P<0.05$) among the non-inoculated than the inoculated heifers. Propionate concentrations in the rumen during days 7 and 8 were lower ($P<0.05$) for non-inoculated animals. Propionibacteria strain P-63 has the necessary attributes concerning ruminal survival and anti-acidotic activity to reduce the potential for acidosis of cattle being adapted to high concentrate diets.
(Key words: Propionibacteria, Acidosis, Heifers, Wheat)

Introduction

Ruminal acute acidosis occurs most frequently when ruminants gain access to large quantities of highly fermentable carbohydrates. This causes extreme shifts in the ruminal environment. Because of microbial changes, lactic acid concentration within the rumen increases causing ruminal pH to decrease. Acidosis also can occur when ruminants are stressed during a rapid adaptation to a high concentrate diet, especially when diets contain high amounts of wheat or rapidly femented corn. This experiment was designed to determine the effects of *Propionibacterium* strain P-63 on cattle stressed by rapidly changing the diet.

Materials and Methods

Ten weanling heifers weighing 270 kg were ruminally cannulated. These heifers were housed individually in stalls at the OSU Nutrition - Physiology Unit in Stillwater, OK. They were randomly divided into two equal groups of five. All animals were ruminally sampled and tested for naturally occurring lactate utilizing bacteria after animals were allotted to treatments. Results of this

screening was not completed until after other segments of the experiment were finished.

The five heifers that were allotted to receive the *Propionibacterium* strain P-63 were pre-inoculated each day for 14 d prior to feeding concentrate. During pre-inoculation all animals were fed grass hay. P-63 inoculum was given once daily in a freeze-dried state packed in 3 gm gelatin capsules throughout the pre-inoculation period and the entire trial. This capsule was placed intra-ruminally in the cannula of the treated heifers every day after the 0 h sample was taken and prior to feeding. Daily dosage of P-63 was set at 3 X 10^{11} cfu per head. This was based on an estimated 30 L capacity rumen so that P-63 was administered at the rate of 1 X 10^{7} cfu/mL of rumen fluid.

All animals were switched from the grass hay diet to a 50% concentrate diet containing ground corn (Table 20) on the first day of the trial. This 50% concentrate diet was fed at 1.5 % of body weigh for the first 5 d of the experiment. On day 6, all animals were fasted for 24 h. After the 24 h fast animals were fed a 90% concentrate diet of which 75% was cracked wheat. This diet was fed for 2 consecutive days at a rate of 2.5% body weight.

During the first 6 d of the trial the heifers were ruminally monitored twice daily at 0 and 8 h post-feeding. On days 7 and 8 when the 90% diet was fed, heifers were sampled intra-ruminally 8X/d (0, 4, 6, 8, 10, 12, 14, and 16 post-feeding). Ruminal parameters monitored during the trial included pH, osmotic pressure, glucose concentrations, and lactate and VFA concentrations.

Ruminal samples were taken using a vacuum pump attached to an intra-ruminally dwelling tube fitted with a stainless steel filter. Approximately 100 mL of fluid was collected at each sampling. A small aliquot (10 mL) was poured off and pH was measured immediately using a combination electrode and a pH meter (Accumet® model 1003 portable pH meter, Fisher Scientific, Pittsburgh, PA). The remaining fluid was centrifuged at 5500 X g for 10 min; the supernatant was decanted and frozen for later analysis for lactate, glucose, VFA, and osmolalities.

VFA and lactate concentrations in the ruminal samples were quantified using a HPLC (Hewlett Packard HPLC system equipped with a diode-array detector; Hewlett Packard, Atlanta, GA). In preparation for HPLC analysis, 0.5 mL of the supernatant was placed into 0.5 mL of 10 mM sulfuric acid. This mixture was filtered through 0.2 um filters directly into 2 mL HPLC autosampler vials. The samples were injected into the HPLC system and concentrations of lactic acid and VFA were calculated based on retention times and peak heights of known external standards measured at 210 nm.

Osmotic pressures of the rumen fluid was determined by freezing point depression analysis (Osmette A - Automatic osomometer model 5002; Precision Systems, Inc., Natick, MA). Glucose concentrations were analyzed using a colormetric assay (Rapid Stat ™ Kit and Glucose Rapid Stat Reagent; Pierce Chemical Co., Rockford, IL.).

Ruminal samples were taken on d 5 to determine establishment of P-63. Samples were taken at 0 h post-feeding but prior to inoculating for the day. These ruminal samples were placed on ice, transported to the Noble Research Center for plating, and plated on a selective media at dilutions of 1 X 10^{-3}, 1 X 10^{-4}, 1 X 10^{-5}, and 1 X 10^{-6}.

Statistical analysis was run using a split-split plot design . Treatment differences were evaluated using a t-test (Steel and Torrie, 1980).

Results and Discussion

Plating on d 5 indicated that all but one of the treated animals had established or maintained a population of propionibacteria P-63 strain. The heifer not showing growth of propionibacteria on d5 on the selective media also had trouble maintaining anti-acidotic conditions within the rumen when the 90% concentrate diet was introduced on d7. Prescreening of animals prior to the onset of the experiment also detected two animals assigned to the control group that had countable naturally occurring lactate utilizers (2.7 X10^{6} and 3.5 X 10^{7} cfu/mL). Based on this information, results form the trial were analyzed statistically using two models; first using all animals in the data base (Model 1)

and second after eliminating the inoculated animal that failed to show growth of propionibacteria and the two animals that exhibited naturally occurring lactate utilizers (Model 2).

Model 1. Ruminal pH of the treated animals on d 8 tended to be higher when compared to those values represented by control animals (Figure 7). On day 7, meann pH values were 5.95 and 5.96, respectively, but on d 8, values were 5.75 and 5.41.

Lactate concentrations also tended to differ (Figure 8). Before d 7, lactic acid was detectable within the rumen of either control or treated animals, but with the onset of the 90% concentrate diet, lactate became detectable in both inoculated and non-inoculated animals being slightly higher for control heifers matching their lower pH values. At the end of d 7 (16 h post-feeding) mean ruminal lactate concentrations were 73 % greater (71.17 mM vs 41.10 mM in non-inoculated heifers than in inoculated heifers. However, this difference is not significant (P=0.54). During d 8 lactate concentrations declined to similar concentrations by 14 h post-feeding. Feed intake was very low on d 8 for both groups, being 0.13 kg and 1.31 kg.

No significant effect of inoculation on ruminal concentrations of VFA (P>0.05) was detected when all heifers were included in the model. Although, propionate concentrations tended to be higher for inoculated animals, especially on d 8. Propionate concentrations for the heifers on d 8 was (37.59 mM vs 43.15 mM). Butyrate concentrations on d 8 tended to be higher for non-inoculated heifers (29.78 mM vs 11.37 mM).

With all animals were included in the model, no treatment effects were significant (P>0.05) for the ruminal parameters, glucose concentration and osmotic pressure.

Model 2. With the two control heifers and the one inoculated animal removed from the data set, inoculation effects became more evident. Although differences in pH values remained nonsignificant, a strong trend (p= 0.08) for higher pH values in inoculated heifers was detected (Figure 9) being 5.30 vs

4.88. Lactate in non-inoculated heifers reached a maximum concentration of 114 mM vs 7.4 mM for inoculated heifers. The mean value for control heifers on d 7 (57.08 mM) was greater ($P<0.05$) than for treated heifers (1.24 mM). Kezar and Church (1979) reported that ruminal pH declined to 4.73 when lactic acid concentration reached 34.2 mM. During 10, 12, 14, and 16 hr post-feeding on d 7, the mean lactate concentrations remained higher ($P<0.01$) for non-inoculated than inoculated heifers (Table 21). Treatment differences on d 8 were not significant ($P=0.14$) but ruminal lactate concentrations remained higher in non-inoculated animals.

Acetate and propionate concentrations on d 7 also were significantly lower ($P<0.01$) for control heifers during 12, 14, and 16 hr post-feeding as shown in Table 22. During d 8 of the trial acetate concentrations remained lower ($P<0.01$) during 0, 4, 6, and 8 hr post-feeding (Table 23) but propionate concentrations were significantly ($P<0.05$) higher for inoculated heifers (48.14 mM vs 29.74 mM). These differences suggest that propionibacteria were utilizing lactate and producing propionate in the inoculated heifers (Hettinga and Reinbold, 1972). Butyrate concentrations also tended to be higher for control heifers (29.19 mM vs 14.72 mM).

Ruminal glucose concentrations tended to be higher in non-inoculated heifers during d 7 of the trial (Figure 10). As shown in this figure, whenever lactic acid accumulated in the rumen of non-inoculated heifers, ruminal glucose concentrations also accumulated. This has been observed previously by Slyter (1976) and Horn et al. (1979). The relationship between lactate and glucose concentrations in the control animals on day 7 was not as strong ($r^2=0.66$) as we detected in our previous acute study. The accumulation of both glucose and lactate in the rumen probably caused osmolality in the rumen to increase during acidosis. The osmotic pressure on d 7 animals was higher ($P<0.01$) at 348 mOsm/kg for non-inoculated than for inoculated animals (315 mOsm/kg).

Implications

Propionibacterium strain P-63 possessed the attributes necessary for ruminal survival and the anti-acidotic activity to reduce the potential for acidosis to occur in cattle being rapidly adapted to high concentrate diets. Because glucose accumulated when lactate accumulated, addition of a microbial strain to prevent glucose accumulation might prove helpful. Some lactobacillis strains have such capacity. Therefore, the efficacy of combining *Propionibacterium* strain P-63 with a glucose-using microbial strain (Lactobacillis) for enhancing performance of cattle being adapted to a diet with high potential to produce acidosis should be evaluated.

Table 20. Composition of the concentrate diets (DM basis).

	Concentrate content	
Ingredient, %	50%	90%
Ground corn	42.2	20.5
Ground wheat		61.5
Cottonseed hulls	20.8	
Alfalfa pellets	29.2	10.2
B-075 supplement[a]	7.8	7.8

[a] Composition of the supplement can be found in table 2 of chapter III.

Figure 7. Ruminal pH values for the heifers in model 1.

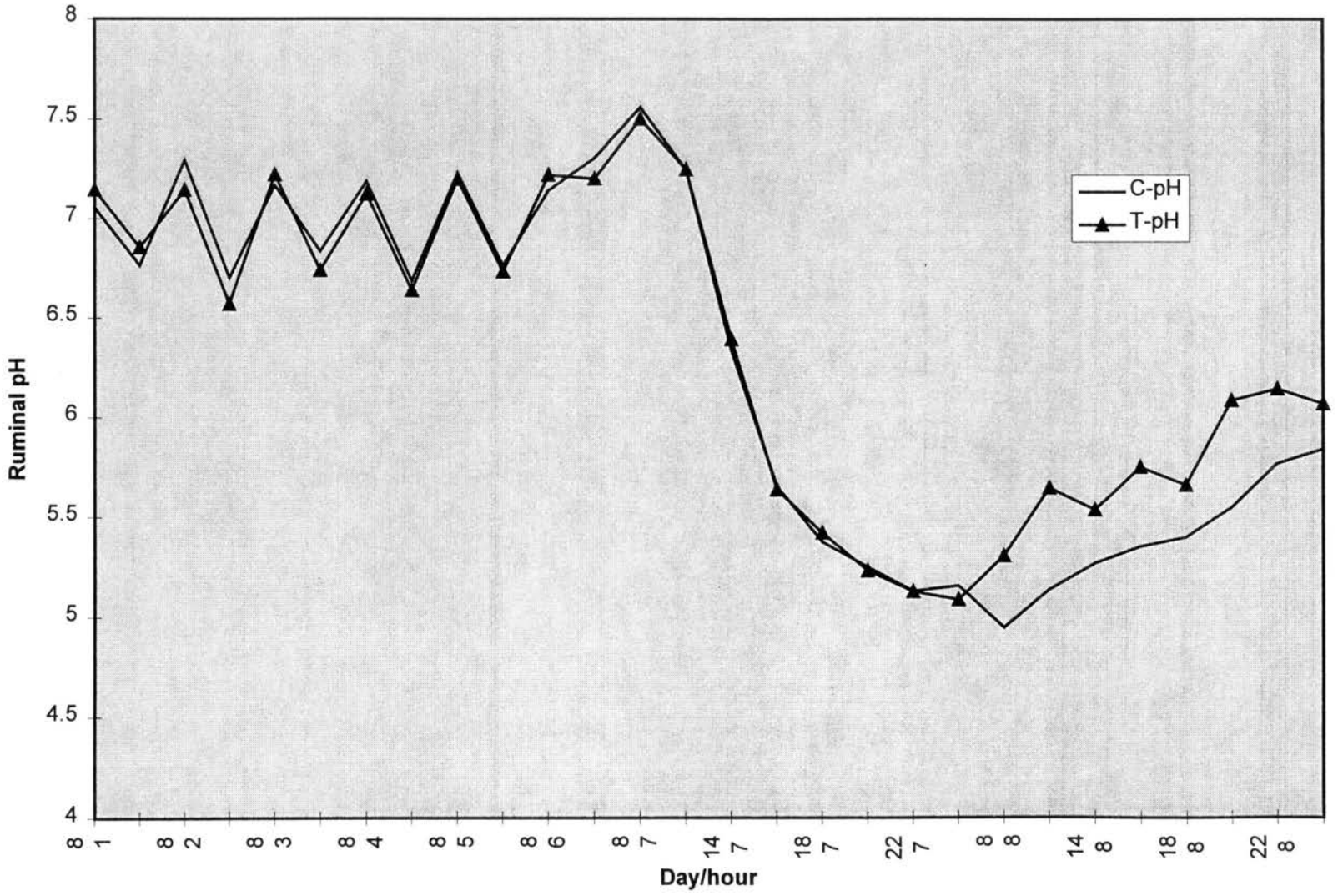


Figure 8. Ruminal lactate concentrations vs ruminal pH in model 1.

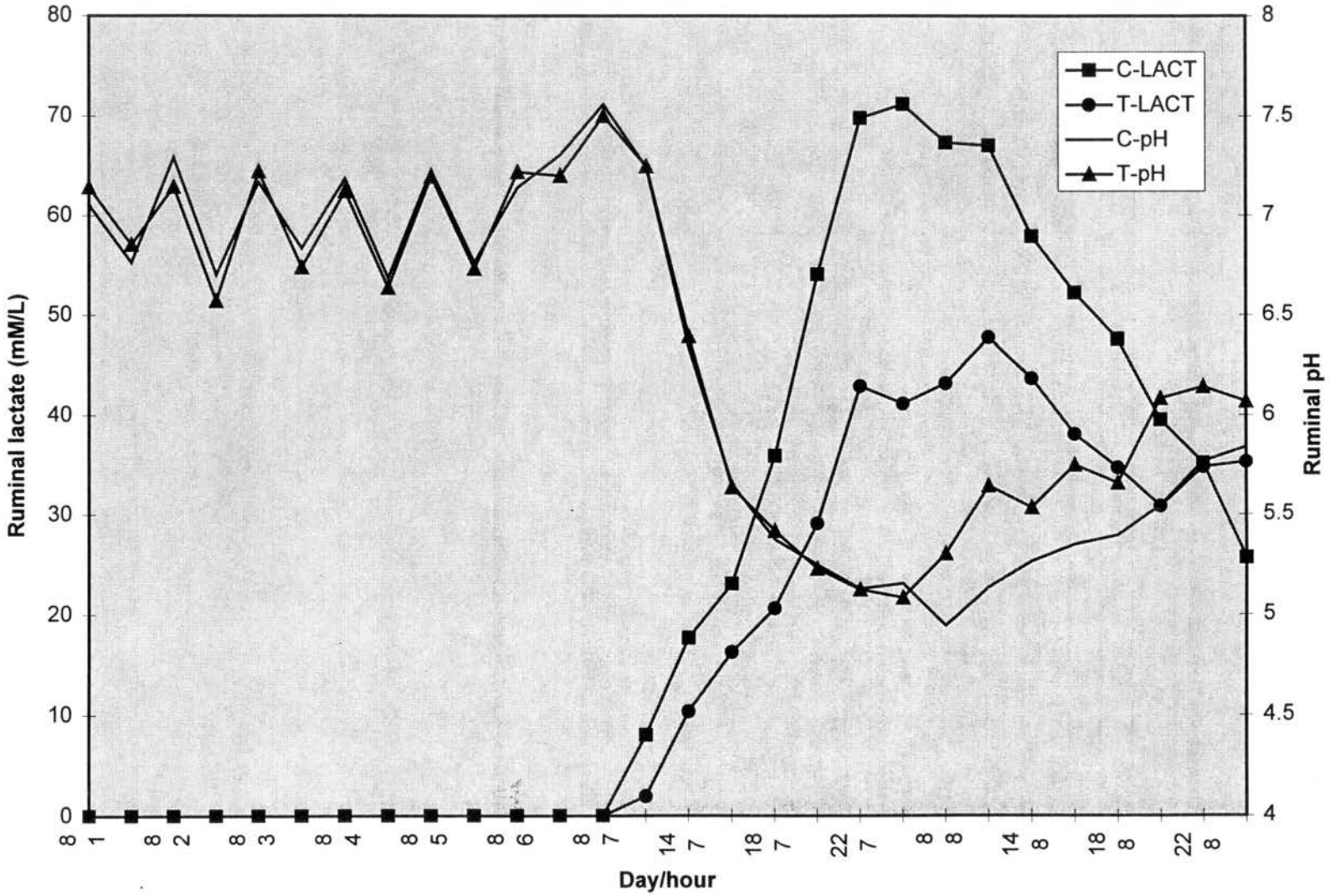


Figure 9. Ruminal lactate concentrations vs. ruminal pH values in model 2.

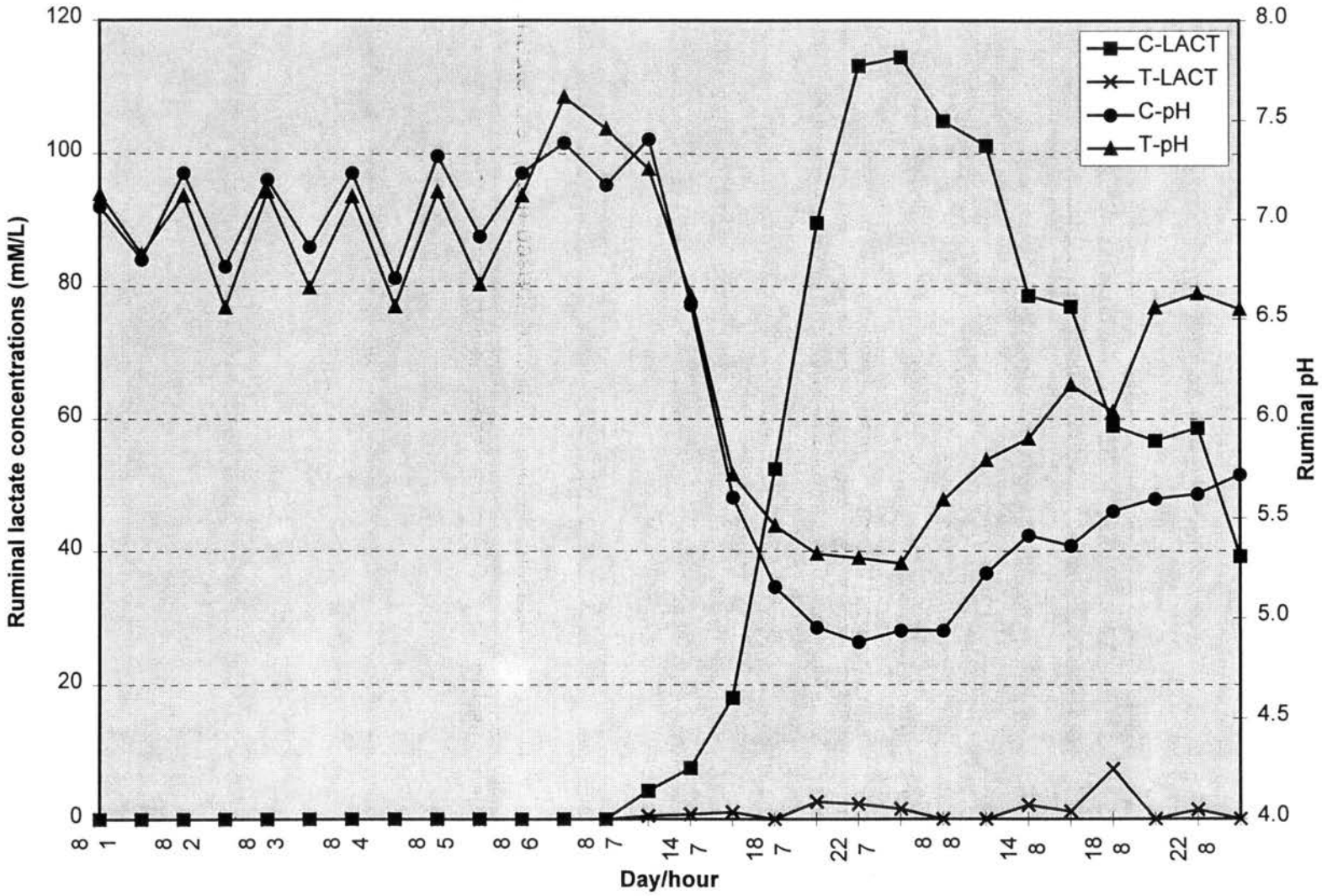


Table 21. Ruminal lactate concentrations during the latter part of day 7 in model 2.

Hour[a]	Lactate concentrations (mM/L)	
	Non-inoculated	Inoculated
10	52.446[b]	0.011[c]
12	89.548[b]	2.614[c]
14	113.191[b]	2.233[c]
16	114.492[b]	1.506[c]

[a]Hours presented as time post-feeding.

[b, c] Mean concentrations in a row with different superscript are significantly different (P<0.01).

Table 22. Ruminal concentrations of acetate and propionate during day 7 in model 2.

Hour[a]	Acetate		Propionate	
	Concentrations (mM/L)			
	Non	Inoc.	Non	Inoc.
12	53.408[b]	69.120[c]	31.446[d]	55.355[e]
14	45.969[b]	68.604[c]	27.909[d]	57.175[e]
16	40.733[b]	66.465[c]	18.545[d]	57.043[e]

[a]Hours presented as time post-feeding.

[b,c,d,e] Mean concentrations in a row with different superscript are significantly different (P<0.01).

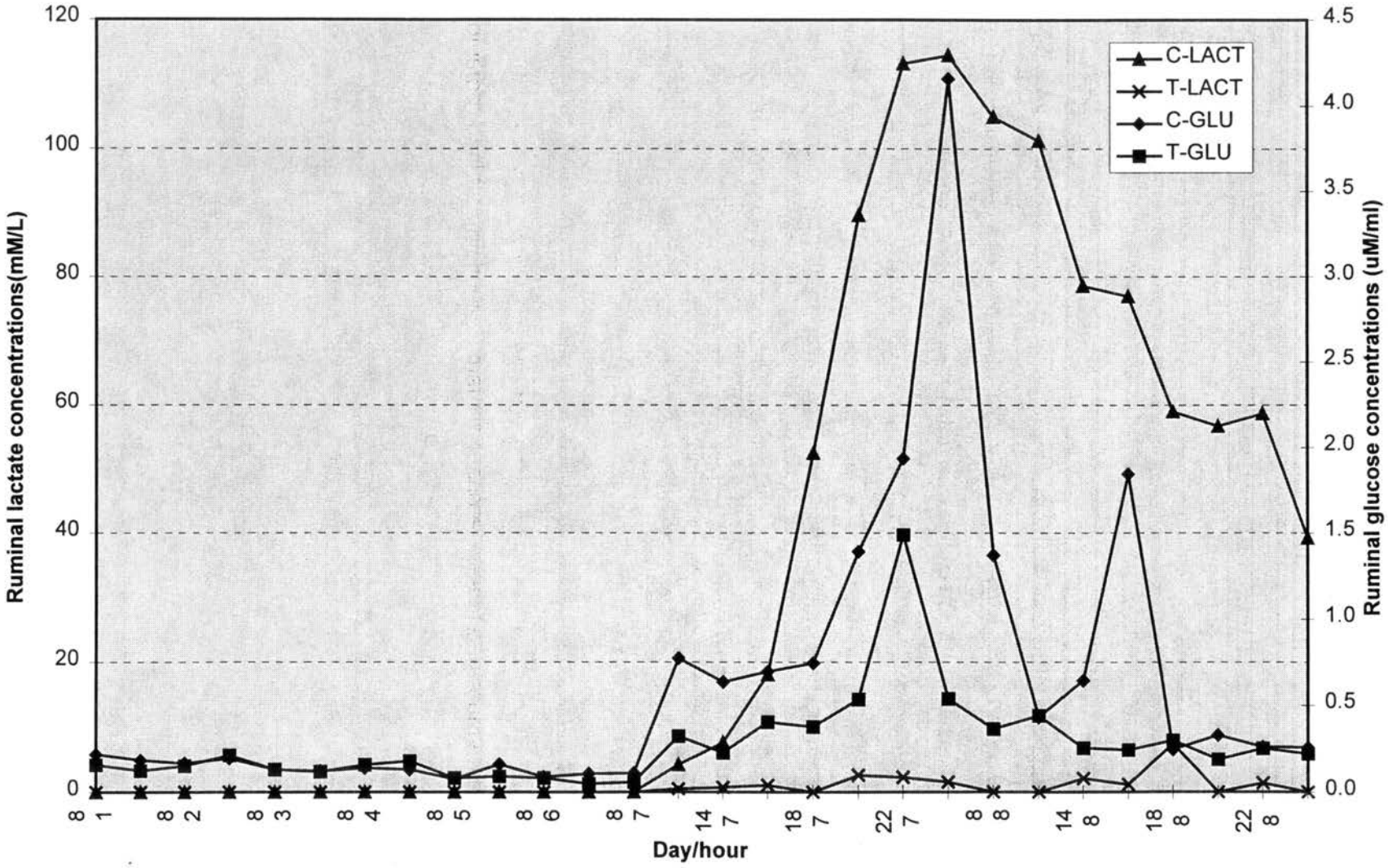
Table 23. Ruminal acetate concentrations during day 8 in model 2.

Hour[a]	Acetate concentrations (mM/L)	
	Non-inoculated	Inoculated
0	19.085[b]	55.063[c]
4	14.925[b]	53.165[c]
6	23.089[b]	49.931[c]
8	29.886[b]	48.722[c]

[a]Hours presented as time post-feeding.

[b, c] Mean concentrations in a row with different superscript are significantly different (P<0.01).

Figure 10. Ruminal glucose concentrations compared to lactate concentrations in model 2.



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CHAPTER VII.

EFFECTS OF INOCULATION WITH EITHER PROPIONIBACTERIA P-63 ALONE OR COMBINED WITH LACTOBACILLI LA53545 ON PERFORMANCE OF FEEDLOT CATTLE

Abstract

Effectiveness of propionibacteria P-63 alone or combined with a lactobacilli on the performance of calves being adapted to a feedlot diet and throughout the feeding period (120 d) was evaluated. Five calves were housed each of 15 pens and five pens (25 calves) received each treatment. The three treatments consisted of 1) no inoculum, 2) propionibacteria P-63 added to the feed at a daily rate of 3.0 X 10^{11} cfu/hd and 3) propionibacteria P-63 (1.0 X 10^{9} cfu/hd) plus lactobacilli LA53545 (1.0 X 10^{8} cfu/hd) each day. Each inocula was poured on top of the ration in the feed bunk. For 14 days, the calves were fed a 50% concentrate diet (corn-based) plus their allotted inoculum. Feed then was withdrawn for 24 hr, after which calves were fed a 90% concentrate diet. To increase the likelihood of acidosis, 75% of the ground corn was replaced by cracked wheat. Although behavior was monitored closely during these 10 days for overt signs of acidosis, no cases were considered serious enough to warrant treatment. Average daily gain (ADG) during these 10 d was greater for calves receiving the combination inoculum than calves receiving the control and P-63 inoculum ($P<0.04$; $P<0.01$). Feed efficiency also was superior for calves receiving the combination inoculum as compared to calves receiving P-63 alone during the first 10 days ($P<0.02$), and during the first 27 days ($P<0.02$). For the total feeding period, feed efficiency for cattle fed the combination inoculum was superior ($P<0.04$) to that of cattle not receiving any inoculum. This response to

inoculation during the early days on trial seems logical because adaptation to grain-rich diets increases the risk of acidosis. The combination inoculant, (propionibacteria plus lactobacilli) presumably by reducing the incidence or severity of acidosis, tended to improve cattle performance.

(Key Words: Acidosis, Propionibacteria, Lactobacilli, Cattle, Feedlot, Wheat)

Introduction

Acidosis occurs in ruminants that consume a large quantity of carbohydrate, particularly grain, particularly when feedlot cattle are being adapted to grain rich diets. To avoid acidosis, the feedlot industry uses a prolonged adaptation time of about 3 weeks. If cattle could be adapted to their high concentrate diet more quickly, cattle should gain more rapidly and the number of days on feed could be decreased. This experiment was designed to determine the effectiveness of inoculating cattle with propionibacteria alone or in combination with lactobacilli both while being adapted to a high concentrate (wheat-based) diet and during the finishing period.

Materials and Methods

Seventy-five calves (mean initial weight 317 kg) were used. These calves, 40 heifers and 35 steers, were housed at the Progeny Barn Oklahoma State University in Stillwater, OK. The heifers and steers were kept separate when allotted to pens with twenty-five calves assigned randomly to each treatment with 5 calves per pen and 5 pens per treatment.

Three treatments were used; these included: 1) no inoculum, 2) propionibacteria P-63 added to the feed (3.0×10^{11} cfu/hd each day) and 3) propionibacteria P-63 (1.0×10^{9} cfu/hd each day) plus lactobacilli (LA53545 included at 1.0×10^{8} cfu/hd each day). The inocula, in a freeze-dried form, was added to 600 mL of warm water which was poured on the daily ration each morning (0800) when fresh feed was added to the bunk.

The calves were fed a 50% corn-based concentrate for 14 d with inoculum dosed daily during this time. Calves then were subjected to a 24 hr fast prior to the introduction of the 90% concentrate diet. Inoculum was dosed orally on the

day of the fast. Diet composition is shown in Table 24. For the first 10 days, the grain portion of this 90% concentrate diet consisted of 75% wheat and 25% corn. Thereafter the concentrate was all cracked corn. Note that neither ionophores nor tylosin were included in these diets

During the 10 days that the cattle were on the 90% wheat-based diet, behavior was monitored closely. Bunk aggressiveness of the calves was monitored every morning during feeding.

Feed intakes were recorded daily during the entire trial. Individual cattle weights were taken on the fasting day to record initial weights for the calves. Calves again were weighed after 10 days on the wheat-based 90% concentrate diet. For the remainder of the 121 d feeding period calves were weighed approximately every 28 d. All weights were adjusted assigning a shrink of 5% for comparison to the initial weight. Average daily gain (ADG) and feed efficiency (FE) were calculated using these data and final 121 d weight which was based on carcass weight.

The calves were transported to Excel, Inc. (Dodge City, KS) for slaughter after 121 d on feed. Hot carcass weights were collected the day of slaughter and final live weight at 121 d was calculated by dividing hot carcass by 0.62. After a 36 h chill, ribeye area (REA), kidney, heart, and pelvic fat percentage (KPH), unadjusted backfat thickness, marbling score, skeletal maturity, lean maturity, and liver abscesses. Dressing percentage (DP), yield grade, quality grade, and overall maturity were calculated.

The pen means were analyzed by using GLM procedure of SAS (1985) with gender and treatment included as classes. Preplanned contrast tested included all inoculations vs. control, comparison of the two inocula, and each separate treatment vs. control.

Results and Discussion

During the 10d when the wheat-based 90% concentrate diet was fed, behavior of the calves was observed; no signs of overt acidosis were serious enough to warrant treatment. No differences between treatments were detected

in bunk aggressiveness. Weights of the cattle are presented in Appendix A. All values prior to carcass weight are live weights of shrunk cattle or of full cattle mathematically shrunk (95% of full weight). Weights on day 121 are hot carcass weights divided by 0.62.

Dry matter intakes are summarized in Table 25. DM intakes for inoculated calves were similar to those calves receiving no inoculum except when averaged across the first 83 d when non-inoculated calves had a higher (P<.02) DM intakes then calves receiving the combination inoculum (9.75 vs. 9.00 kg/d).

Performance results are presented in Table 26 from day 0 to day 121. The NE_m, NE_g and ME values calculated based on performance data from day 0 to day 121; no treatment differences were significant.

ADG during the 10d during which the wheat-based 90% concentrate diet was fed was greater (P<.04) for the calves receiving the combination inoculum than for calves receiving P-63 alone (1.63 vs. .94 kg/d). For the remainder of the feeding period, no differences between treatments were significant.

During the days in which the cattle were fed the wheat-based diet, the calves given the combination inoculum had a superior (P<.02) efficiency to calves given the propionibacteria P-63 alone. Calves receiving the combination inoculum tended (P<.06) to be more efficient than control calves (4.47 vs. 7.26). This improved efficiency for the combination inoculum remained on day 27 (P<.05) when compared to both the control and P-63 treatments. Calculated over the entire feeding period and carcass-adjusted final weight, calves fed the P-63 plus lactobacillus combination inoculum were more efficient (P<.04) than cattle receiving no inoculum.

Calves receiving the combination inoculum had feed/gain ratios that were 7% better than calves receiving no inoculum and 4 % than those receiving only P-63. These efficiency improvements are comparable to those obtained from ionophores (Goodrich et al., 1984; Stock et al., 1995).

The impact of inoculation on carcass characteristics are presented in Table 27. No differences (P>.05) were noted for any of the carcass variables

measured. Similarly, feeding ionophores does not alter carcass characteristics of feedlot cattle (Spires et al., 1990; Galyean et al., 1992). Final yield grades averaged 2.78. Of the control and combination cattle, 34% graded choice or prime, with 66% grading select. Of the calves fed P-63, 16% graded choice or prime, with the remainder grading select (P<.16).

Implications

Cattle continuously receiving a combination inoculant (lactobacilli and propionibacteria) had feed intakes and ADG similar to non-inoculated cattle but feed efficiency was improved by 7 by the combination inoculation. The combination of *Propionibacterium* P-63 and *Lactobacillus* LA53545 appears to have value as an inoculant, especially for the adaptation period, presumably by reducing the acidosis incidence or severity. The repeatability of these treatment effects across diverse types of cattle needs testing before one can advocate inoculation of typical feedlot cattle with this combination inoculum. More research is needed on (1) need for preliminary dosing with the inoculum, (2) alternative administration methods such as pastes or via micro-ingredient machines, (3) relative benefit from lactobacillus alone, (4) need for feeding the inoculum beyond the adaptation period and (5) liver abscess incidence.

Table 24. Composition of the concentrate diets (DM basis).

	Concentrate content		
Ingredient, %	50%	90%	
		Wheat	Corn
Ground corn	42.2	20.5	82.0
Ground wheat		61.5	
Cottonseed hulls	20.8		
Alfalfa pellets	29.2	10.2	10.2
B-075 supplement[a]	7.8	7.8	7.8

[a] Composition of the supplement can be found in table 2 of chapter III.

Table 25. Intakes for the cattle receiving no inoculum, P-63 alone, or a combination inoculum (dry matter/day).

Feed intake, kg. DM/d	Control	P-63	P-63 & Lacto
days 0-10	7.19	7.07	7.05
days 0-27	9.25	8.75	8.96
days 0-83	9.75[a]	9.00[a]	9.30[b]
days 84-119	10.32	9.84	10.45
days 0-121	10.00	9.32	9.72

[a,b] Means within a row without common superscripts differ, $P<0.05$.

Table 26. The performance summary for the cattle receiving no inoculum, P-63 alone, or a combination inoculum.

Performance Item	Control	P-63	P-63 & Lacto	SE
Calculated Net Energy Values				
ME, Mcal/kg	2.86	2.87	2.96	0.09
NE_m, Mcal/kg	1.95	1.96	2.03	0.08
NE_g, Mcal/kg	1.30	1.31	1.37	0.07
ADG, kg				
days 0-10	1.11[a]	0.93[a]	1.63[b]	0.31
days 0-27	2.00	1.94	2.12	0.07
days 0-83	1.88	1.87	1.81	0.08
days 84-121	1.47	1.37	1.51	0.19
days 0-119	1.98	1.94	1.94	0.10
days 0-121	1.89	1.88	1.88	0.10
FE, feed/gain (DM basis)				
days 0-10	7.26	8.19	4.47[c]	2.15
days 0-27	4.62[a]	4.68[a]	4.14[b]	0.33
days 0-83	5.21	4.98	4.99	0.25
days 84-119	7.18	7.79	6.66[d]	0.79
days 0-119	5.07	5.01	4.82	0.23
days 0-121	5.32	5.17	4.97[e]	0.23

[a,b] Means within a row without common superscripts differ, $P<0.05$.

[c] Mean is different from P-63, P=.02.

[d] Mean is different from P-63, P=.04.

[e] Mean is different from control, P=.04.

Table 27. Impact of inoculation on carcass characteristics.

Carcass measure	Control	P-63	P-63 & Lacto
Dressing percentage	61.26	61.61	61.63
Ribeye area, cm^2	88.9	89.4	87.9
Ribeye area, cm^2/cwt carcass	11.57	11.92	11.64
Kidney, pelvic & heart fat, %	2.17	2.20	2.30
Unadjusted backfat, cm	1.22	1.14	1.19
Adjusted backfat, cm	1.42	1.30	1.42
Skeletal maturity	153	155	152
Lean maturity	141	155[b]	144
Overall maturity	147	155	148
Liver abscesses, %	8	8	0
Preliminary yield grade	2.54	2.44	2.54
Final yield grade	2.84	2.63	2.87
Marbling Score[a]	312	309	335
Quality grade	1.48	1.32	1.44
Choice or prime grade, %	32	16	36
Select grade, %	68	84	64

[a] slight amount of marbling (200-299); small amount (300-399).

[b] Mean is different from control, P=.01.

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CHAPTER VIII.

SUMMARY

Acidosis during the period of diet adaptation retards performance and can have permanent effects on performance and liver abscess incidences and severity. Conventional management practices such as narrow-spectrum antibiotics, feed intake control, controlled grain processing, and alterations in roughage sourceand level have helped to reduce the incidence of acidosis. Inoculation also may prove useful. A propionibacteria strain is now being used as a direct fed microbe to prevent nitrate toxicity. Other strains of propioinibacteria can utilize lactate may help to prevent acidosis.

Co-cultures of 3 or 5 strains of propionibacteria were tested in cattle during short adaptation from roughage to concentrate diets in the first four trials. These strains failed to become established in the rumen. Perhaps these different strains were inhibiting each other; subsequent trials we used one propionibacteria strain (P-63) alone.

An acute study was lasting 14 hours conducted to acquire a deeper understanding of acidosis. Steers were intra-ruminally dosed with a starch slurry and fed fine ground corn. Ruminal pH dropped drastically (7.4 to 4.8) between 8 and 12 hours post-engorgement. Lactate concentrations in the rumen were increased markedly at 12 and 14 h post-engorgement when it reached a mean concentration of 163 mM. Ruminal concentrations of lactate and glucose were correlated (R^2=.98).

The next trial determined the efficacy of propionibacteria strain P-63 for controlling acidosis. Ten heifers were rapidly adapted to a 75% wheat-based diet. The inoculated heifers had greater ability to maintain normal ruminal pH and prevent lactate from accumulating. Ruminal conditions of non-inoculated

heifers reached lactate concentrations of 114 mM, pH of 4.88 and concentrations of glucose during acidosis of 4.16 mM. Glucose-utilizing bacteria as a co-inoculant might help control acidosis.

The feedlot phase of the research was designed to test the efficacy of using propionibacteria strain P-63 alone or in conjunction with lactobacilli strain LA53545 for cattle being rapidly adapted to a wheat-based diet. This wheat-based diet was fed for 10 days after which cattle were fed a corn-based diet for a total of 121 d. Cattle continuously receiving the combination inoculant (propionibacteria plus lactobacilli) had a faster rate of gain than non-inoculated cattle during the adaptation period and overall feed efficiency was improved by 7 by inoculation.

Feeding the combination of *Propionibacteria* strain P-63 and *Lactobacllus* LA 53545 continuously, especially during adaptation to a concentrate diet, presumably by reducing the incidence or severity of acidosis, and appears to improve performance of feedlot cattle.

APPENDIX

APPENDIX A

AVERAGE WEIGHTS FOR CALVES IN THE FEEDLOT TRIAL

RECEIVING NO INOCULUM, P-63 ALONE, OR A COMBINATION INOCULUM

Weight, kg.	Control	P-63	P-63 & Lacto	SE
initial	335	322	326	8.1
days 10	346	332[a]	343	8.5
days 27	389	375	384	8.6
days 83	516	503	501	11.7
days 119	570	553	557	15.9
carcass wt	349	341	343	9.9
final weight (carcass wt/.62)	563	550	554	15.9

[a]Mean weight is different (P=0.05) than the control weight in this row.

2

VITA

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Doctor of Philosophy

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