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Ruminal Acidosis in Beef Cattle: The Current Microbiological and Nutritional Outlook[1,2]

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ABSTRACT

Ruminal acidosis continues to be a common ruminal digestive disorder in beef cattle and can lead to marked reductions in cattle performance. Ruminal acidosis or increased accumulation of organic acids in the rumen reflects imbalance between microbial production, microbial utilization, and ruminal absorption of organic acids. The severity of acidosis, generally related to the amount, frequency, and duration of grain feeding, varies from acute acidosis due to lactic acid accumulation, to subacute acidosis due to accumulation of volatile fatty acids in the rumen. Ruminal microbial changes associated with acidosis are reflective of increased availability of fermentable substrates and subsequent accumulation of organic acids. Microbial changes in the rumen associated with acute acidosis have been well documented. Microbial changes in subacute acidosis resemble those observed during adaptation to grain feeding and have not been well documented. The decrease in ciliated protozoal population is a common feature of both forms of acidosis and may be a good microbial indicator of an acidotic rumen. Other microbial factors, such as endotoxin and histamine, are thought to contribute to the systemic effects of acidosis. Various models have been developed to assess the effects of variation in feed intake, dietary roughage amount and source, dietary grain amount and processing, step-up regimen, dietary addition of fibrous byproducts, and feed additives. Models have been developed to study effects of management considerations on acidosis in cattle previously adapted to grain-based diets. Although these models have provided useful information related to ruminal acidosis, many are inadequate for detecting responses to treatment due to inadequate replication, low feed intakes by the experimental cattle that can limit the expression of acidosis, and the feeding of cattle individually, which reduces experimental variation but limits the ability of researchers to extrapolate the data to cattle performing at industry standards. Optimal model systems for assessing effects of various management and nutritional strategies on ruminal acidosis will require technologies that allow feed intake patterns, ruminal conditions, and animal health and performance to be measured simultaneously in a large number of cattle managed under conditions similar to commercial feed yards. Such data could provide valuable insight into the true extent to which acidosis affects cattle performance.
Key words: acidosis, cattle, organic acid, ruminal microbe

INTRODUCTION

The reticulorumen is an anaerobic ecosystem in which microbial digestion of feedstuffs converts fermentable substrates mainly into organic acids, which are then removed primarily by absorption. As long as substrate availability is not excessive and the rate of absorption keeps up with production, ruminal fermentation is stable and mean ruminal pH is generally higher than 5.5, often in the range of 5.8 to 6.5 in grain-adapted cattle. Ruminal pH fluctuates considerably in a 24-h period and is influenced by the intake of fermentable carbohydrate, inherent capacity of the animal to provide buffer, and rates of utilization and absorption of acids. In beef cattle fed high-concentrate diets, the ability of the animal to buffer the rumen is limited by inadequate salivary secretion. If the absorptive capacity of the ruminal wall is impaired by abnormal ruminal papillae or rumenitis, then the animal's ability to maintain a stable ruminal pH is affected. However, when ruminal pH drops below 5.6, VFA absorption is enhanced because VFA become more protonated or undissociated (pKa ~4.9 for VFA), which increases absorption rate (Bergman, 1990). The advantage of rapid absorption may be offset by a shift in microbial populations toward lactic acid production, which will further reduce

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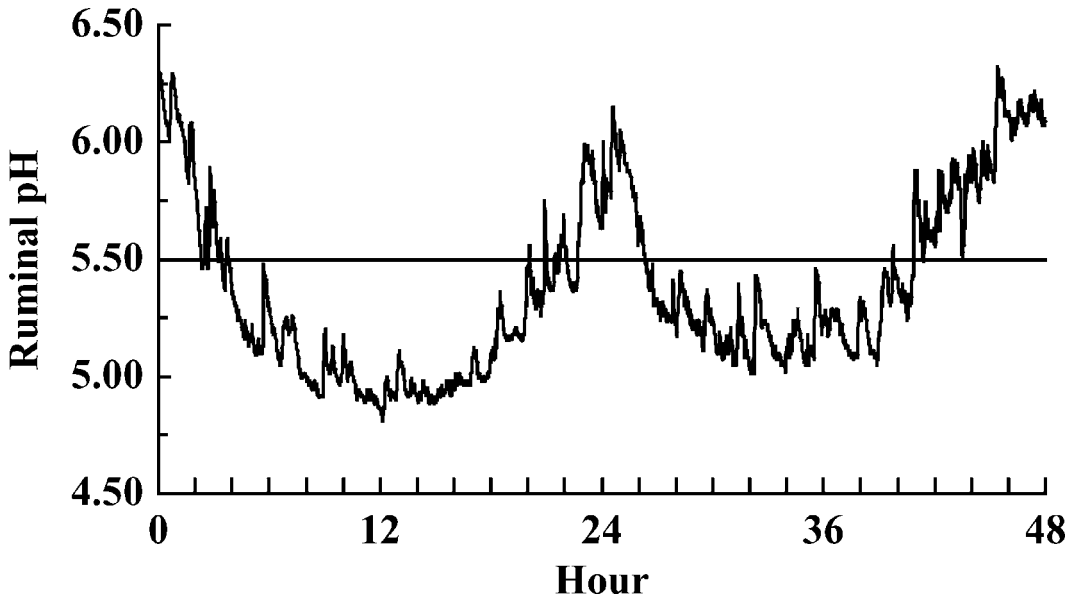

Figure 1. Ruminal pH over a 48-h period of a steer adapted to a 92.5% concentrate diet based on dry-rolled corn fed once daily at 0 and 24 h. Ruminal pH was monitored with a submersible pH electrode suspended through the plug of the ruminal cannula and was recorded every minute (data from Cooper et al., 1998).

ruminal pH. Lactic acid is about 10 times stronger than VFA (pKa 3.9 vs. 4.9); hence, it is less protonated than VFA, and thereby accumulates in the rumen and contributes to the downward spiral in ruminal pH. Of the 2 isomers of lactic acid, L(+) lactate is the predominant isomer in the rumen and the proportion of D(−) lactate generally increases with lower pH (Giesecke and Stangassinger, 1980). However, the ruminal ratio of the 2 isomers is not reflective of production because of interconversion catalyzed by lactate racemase, which is produced by certain lactate-fermenting bacteria (Asanuma and Hino, 2002b). There may also be a difference in the rates of absorption of the 2 isomers with net portal absorption of L(+) lactate generally being greater than that of D(−) lactate (Harmon et al., 1985).

Ruminal pH is a critical factor in the normal and stable function of the rumen because of its profound effect on microbial populations and fermentation products, and on physiological functions of the rumen, mainly motility and absorptive function. Therefore, nonphysiological accumulation of organic acids and consequent reduction in pH below the normal (<5.6) has significant impact on microbial activity, rumen function, and animal productivity and health. Reduction in postprandial ruminal pH by as much as 1.0 unit, which is a 10-fold increase in hydrogen ion concentration, is not uncommon. An example of diurnal ruminal pH pattern monitored continuously over a 2-d period in a steer fed a 92.5% concentrate diet based on corn grain once daily is shown in Figure 1 (Cooper et al., 1998). In beef cattle fed high-grain diets, ruminal pH can range from 6.5 to 5.6, with average pH typically around 5.8 to 6.2, but it can drop below 5.6 for a period during the feeding cycle. What is not known is the minimum time of suboptimal pH needed to have a detrimental effect on microbial activity, nutrient digestion, and ruminal function. In vitro simulation systems, particularly dual-flow continuous culture systems, have been used to assess mean pH, diurnal variation, fluctuations in pH, and duration of suboptimal pH on microbial activity, nutrient digestion, and nutrient flow (Lana et al., 1998; Russell, 1998; de Veth and Kolver, 2001; Calsamiglia et al., 2002; Yang et al., 2002; Wales et al., 2004), although most of these studies were designed to evaluate pH effects on fermentation of dairy diets. de Veth and Kolver (2001) examined the effect of duration of suboptimal pH on nutrient digestion and microbial protein synthesis in a dual-flow continuous culture system with rye grass as the substrate. A suboptimal pH of 5.4 was maintained for 0, 4, 8, and 12 h during a 24-h period, and the results showed a significant linear reduction in VFA production and microbial N flow. A similar study with grain as the substrate with microbial inoculum from a grain-adapted animal would be more relevant to subacute acidosis in beef cattle. When assessing the significance of ruminal pH, it is important to consider not only the mean pH, but also the postprandial fluctuations, particularly the duration of suboptimal pH (<5.6).

Ruminal acidosis results when cattle consume fermentable carbohydrates in amounts sufficient to cause

Table 1. Comparison of acute and subacute acidosis in beef cattle[1]

Item	Acidosis	
	Acute[2]	Subacute[3]
Clinical signs	Present	Absent
Mortality	Yes	No
Ruminal changes		
Fermentation products		
Ruminal pH	<5.0	5.0 to 5.5
Total organic acids	Increased	Increased
Lactic acid	High (50 to 120 m*M*)	Normal (0 to 5 m*M*)
VFA	Below normal (<100 m*M*)	High (150 to 225 m*M*)
Microbes		
Gram-negative bacteria	Decreased	No change
Gram-positive bacteria	Increased	No change
Streptococcus bovis	Increased initially	No change
Lactobacillus spp.	Increased	Increased
Lactic acid-producers	Increased	Increased
Lactic acid-utilizers	Decreased	Increased
Ciliated protozoa	Absent or decreased	Absent or decreased
Microbial "toxic" products		
Ethanol	Increased	ND[4]
Amines	Increased	ND
Endotoxins	Increased	Increased
Blood changes		
pH	Decreased (<7.350)	Normal to slightly decreased
Lactic acid	Increased, particularly D(–)	Normal
Bicarbonate	Marked reduction (<20 mEq/L)	Normal to transient reduction
Base status	Base deficit	Base excess
Packed cell volume	Increased (>40%)	Normal (30 to 35%)
Endotoxins	Yes	Yes
Inflammatory mediators	Yes	Yes
Sequelae		
Rumenitis	Yes	Yes
Laminitis	Yes	Yes
Polioencephalomalacia	Yes	Yes
Liver abscesses	Yes	Yes

[1]Data from Nagaraja et al. (1998).

[2]Changes in response variables are in relation to forage-adapted animal.

[3]Changes in response variables are in relation to grain-adapted animal.

[4]ND = not determined.

nonphysiological accumulation of acids in the rumen with a concurrent reduction in pH. Ruminal acidosis has been well known ever since grain feeding became a common practice, and it is considered as the most common nutritional disorder in feedlot cattle. Britton and Stock (1989) recognized that "acidosis is not one disease, but rather a continuum of degrees of ruminal acidity" and for simplicity it is categorized as acute or subacute based on ruminal pH, type of acid responsible for low pH, and whether clinical signs are evident (Table 1).

Ruminal Acidosis, pH, and Concentrations of Lactic Acid and VFA

Because ruminal acidosis represents varying degrees of acidity in the rumen, it is well accepted that total organic acid (VFA and lactic acid) accumulation dictates whether the rumen is acidotic (Britton and Stock, 1989; Owens et al., 1998). Ruminal pH of 5.6 or below is generally considered the benchmark for ruminal acidosis; a pH range of 5.0 to 5.6 is regarded as subacute or chronic acidosis; and pH below 5.0, approaching 4.5 or lower, is considered acute acidosis (Britton and Stock, 1989; Owens et al., 1998; Krause and Oetzel, 2006). In acute acidosis, the reason for pH to reach 4.5 or below is lactic acid accumulation, which is the result of increased lactic acid production and decreased lactic acid fermentation (Figure 2A). The total VFA concentration generally increases at the onset of acidosis, but with progression of acidosis VFA concentrations decline dramatically because of destruction of the normal bacterial flora and ruminal dilution from influx of fluids to compensate for increased osmolality (Huber, 1976). The increased production is essentially because of the establishment of an acid-tolerant lactobacilli population, and decreased fermentation is because the pH is below the range necessary for the lactate-fermenting

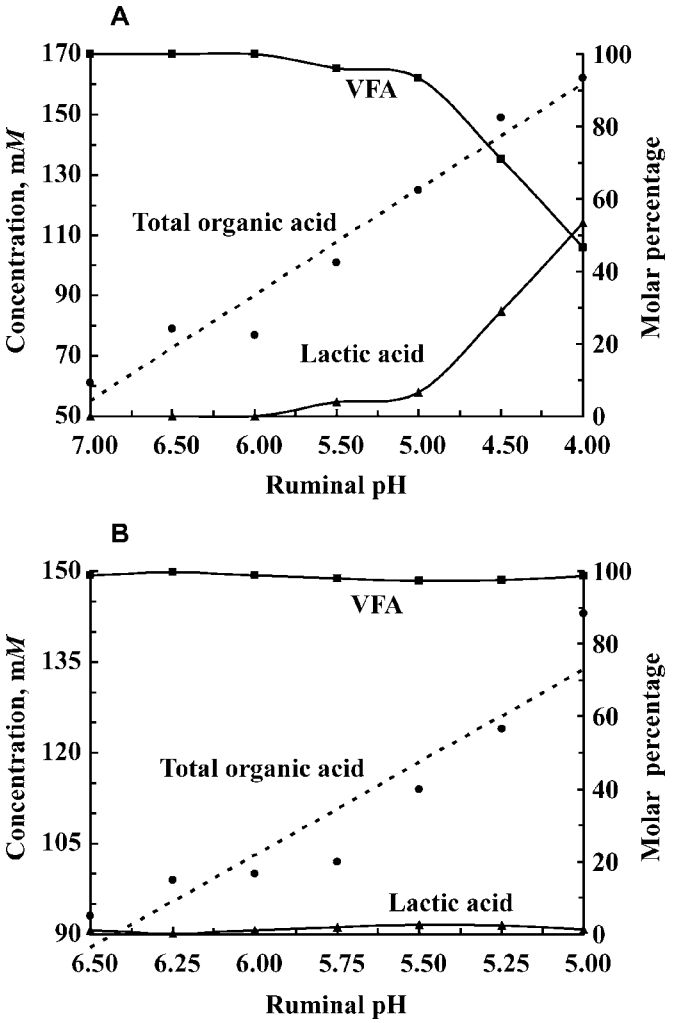

Figure 2. Total organic acid concentrations (●) and molar proportions of total VFA (■) and lactic acid (▲) in A) acute acidosis (data from Nagaraja et al., 1985) and B) subacute acidosis (data from Goad et al., 1998).

bacteria to be active (Therion et al., 1982). In subacute acidosis, the reason for pH to drop below 5.6 is accumulation of VFA, which is a combination of overproduction (increased substrate) and possibly decreased absorption. Although lactic acid is produced during subacute acidosis, it does not accumulate because lactate-fermenting bacteria remain active (Goad et al., 1998) and rapidly metabolize it to VFA (Figure 2B). As the pH nears 5.0 or below for a sustained period, the growth of lactate-fermenting bacteria is inhibited, and hence lactate begins to accumulate. Therefore, subacute acidosis has the potential to become lactic acidosis if the pH of 5.0 is sustained for a time. However, the length of time necessary to make that happen has not been determined.

The topic of acidosis in beef cattle has been reviewed extensively (Dunlop and Hammond, 1965; Dirksen, 1970; Dunlop, 1972; Counotte and Prins, 1981; Britton and Stock, 1989; Nocek, 1997; Owens et al., 1998; Krause and Oetzel, 2006) in the literature. However, a review of microbial changes associated with the onset of ruminal acidosis has not been published since 1976 (Slyter, 1976). In addition, much of our knowledge on ruminal and systemic changes associated with acidosis is based on experimentally induced acidosis in cattle and sheep. A critical evaluation of various methods of induction and models of acidosis is not available. Therefore, this review addresses the microbial changes associated with ruminal acidosis and provides a perspective on the experimental models developed to study acidosis in beef cattle.

THE CURRENT MICROBIOLOGICAL OUTLOOK

Robert E. Hungate, considered the father of rumen microbiology, was the first to study alterations in the microflora of the rumen to explain the "microbial actions" causing acid indigestion in sheep and cattle. He and his colleagues (Hungate et al., 1952) reported that an excess of grain or glucose introduced into the rumen caused a "marked change" in the rumen microbial flora. The changes observed included the following: the cellulolytic bacteria were greatly decreased in numbers; the protozoa were killed; the relative numbers of gram-positive bacteria increased; nonvolatile acids accumulated; and the concentrations of volatile acids diminished. The study also presented evidence that *Streptococcus bovis*, a gram-positive organism, was the major cause of ruminal acidity. Remarkably, even after 5 decades of progress in rumen microbiology, the initial observations on major microbial alterations during ruminal acidosis remain valid. An intriguing observation reported by Hungate et al. (1952) was that the amount of grain capable of inducing acute indigestion in hay-fed animals caused no ill effects if the animals were gradually accustomed to the amount. Although the existence of lactate-utilizing bacteria was known (Mackenzie, 1967) at the time, the relationship between grain adaptation and an increase in the population of bacteria that utilize lactate was not known. Evidence that a change or adaptation in the ruminal microbial population is responsible for tolerance to grain was published by Allison et al. (1964a). They reported that lambs inoculated intraruminally with ruminal contents from sheep that had been adapted to a wheat diet did not get as sick as control lambs following feeding of cracked wheat through the ruminal cannula.

Increased availability of fermentable carbohydrate stimulates growth rates of all microbes resulting in

an overall increase in the rate of fermentation and an increase in end-product production. Among the ruminal microbes, only bacteria and ciliated protozoa are the major participants in the increased fermentation rate associated with grain feeding. Species of ruminal fungi do have amylase activity (Mountfort and Asher, 1988) and are capable of digesting starch in cereal grains (McAllister et al., 1993), but because their numbers are reduced in grain-fed animals (Obispo and Dehority, 1992), it is generally believed that ruminal fungi have a minimal to insignificant role in grain-fed animals.

Almost all studies that have monitored microbial changes in relation to acidosis, with a few exceptions (Krogh, 1963a,b; Braun et al., 1992), have been carried out with experimentally induced acute or subacute acidosis. Microbial changes associated with increased lactic acid production and accumulation have been well documented (Dirksen, 1970; Dunlop, 1972; Slyter, 1976), but not much is known about changes resulting from subacute acidosis (Goad et al., 1998).

Ruminal Acidosis and Bacterial Changes

Ruminal bacteria respond to increased availability of fermentable substrates, such as starch and sugars, by increasing growth rates and fermentative activities. This situation leads to increased production of VFA, and, as long as absorption from the rumen keeps up with production, ruminal pH (5.6 to 6.5) and VFA concentrations (80 to 170 m*M*) are within the normal range and ruminal activities and functions remain normal and stable. Bacterial changes associated with ruminal acidosis primarily include shifts in the populations of starch- and soluble sugar-fermenting bacteria (amylolytic, maltose-, and glucose-fermenting bacteria) and lactic acid-fermenting bacteria.

Amylolytic and Lactic Acid-Producing Bacteria. Many species of ruminal bacteria actively degrade starch and utilize the intermediate products (amylodextrins, maltose, and glucose; Figure 3). The proportion of amylolytic bacteria in the rumen can be as high as 90 to 95% of total culturable bacteria in grain-fed animals (Leedle and Hespell, 1980). The predominant amylolytic, amylodextrin-, and maltose-utilizing bacteria in the rumen include species of *Bifidobacterium*, *Butyrivibrio*, *Eubacterium*, *Lactobacillus*, *Mitsuokella*, *Prevotella*, *Ruminobacter*, *Selenomonas*, *Streptococcus*, *Succinimonas*, and *Succinivibrio* (Kotarski et al., 1992; Chesson and Forsberg, 1997; Stewart et al., 1997). The relative contribution of each genus or species to the overall amylolytic activity and production of lactic acid and VFA is not known. Mackie and Gilchrist (1979) reported that *Butyrivibrio*, *Eubacterium*, and *Lactobacillus* were the major genera in sheep adapted to a high-grain diet. Tajima et al. (2001) used real-time quantitative PCR for specific detection and quantification of 13 species of ruminal bacteria in the rumens of cows switched from a hay to a grain diet. Among the amylolytic and soluble sugar-fermenting bacteria, the concentrations of *Prevotella bryantii*, *Selenomonas ruminantium*, and *Mitsuokella multiacidus* increased, whereas those of *Strep. bovis*, *Eubacterium ruminantium*, *Succinivibrio dextrinosolvens*, and *Treponema bryantii* declined.

Among amylolytic bacteria, *Ruminobacter amylophilus*, *S. ruminantium*, and *Strep. bovis* exhibit the highest growth rates and amylolytic activities (Cotta, 1988, 1992; McAllister et al., 1990). *Bifidobacterium* species (*adolescentis*, *boum*, *globosum*, *merycicum*, *ruminale*, *ruminantium*, *thermophilum*, etc.) are gram-positive rods that have been isolated from the rumens of animals fed starch-based diets (Scardovi et al., 1969; Biavati and Mattarelli, 1991). These organisms do not use starch but can metabolize maltose and glucose. The organism can use a variety of soluble sugars, and have an uncommon pathway (fructose-6-phosphate shunt or bifid pathway) to ferment glucose to acetic and lactic acids in the molar ratio of 3:2 (Scardovi et al., 1969). *Butyrivibrio* isolates are gram-negative rods with gram-positive-like cell wall ultrastructure, and they represent a significant proportion of the rumen bacterial population. Although they are considered fibrolytic organisms, some strains have amylase activity (McAllister et al., 1990) and can produce large amounts of lactic acid from glucose (Van Gylswyk, 1977; Marounek and Bartos, 1987). *Succinimonas amylolytica*, a gram-negative rod, is normally associated with starch digestion and produces mainly succinate and some acetate and formate (Bryant et al., 1958) from glucose. *Prevotella* (*bryantii*, *brevis*, *albensis*, and *ruminicola* spp.) is one of the most numerous genera of the rumen (Bryant et al., 1958) and several strains have amylase activity (Avgustin et al., 1994). *Ruminobacter amylophilus*, a gram-negative rod, is a major amylolytic species in the rumen of grain-fed animals. However, it has a very limited substrate range, utilizing only starch, amylodextrins, and maltose and is unable to use free glucose (Cotta, 1988), possibly because of its inability to transport glucose into the periplasmic space (Anderson, 1995).

Ruminal bacteria that have fast growth rates, rapidly ferment starch or soluble sugars, and could contribute to rapid accumulations of DL-lactic acid and VFA include *S. ruminantium*, *Strep. bovis*, and anaerobic lactobacilli. *Selenomonas ruminantium*, a gram-negative curved rod, is a predominant species in the rumen and consistently increases in concentration in grain-fed animals; it may be the most dominant organism in animals

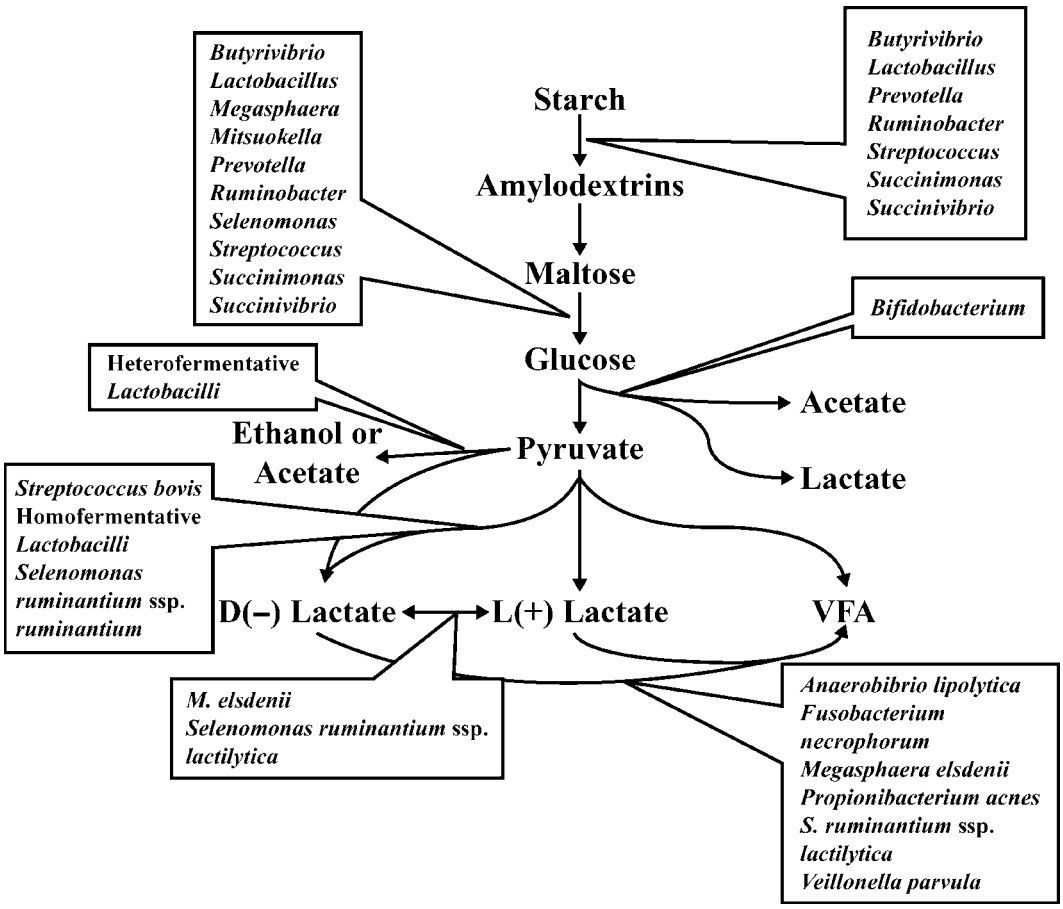

Figure 3. Amylolytic, maltose-fermenting, glucose-fermenting, and lactic acid-fermenting bacteria involved in starch fermentation to lactic acid and VFA in the rumens of grain-fed cattle.

adapted to high-grain diets (Caldwell and Bryant, 1966; Latham et al., 1971). The organism does not ferment starch or other polysaccharides, but uses maltose, sugars, and some oligosaccharides; hence, it is dependent on cross feeding from polymer-degrading bacteria (Ricke et al., 1996). Ruminal selenomonads are classified into 2 subspecies, *ruminantium* and *lactilytica*, based mainly on their ability to utilize lactate and glycerol (Ricke et al., 1996). Strains that utilize lactate and glycerol are placed in the subspecies *lactilytica*, and all other strains are grouped under the subspecies *ruminantium*. Therefore, *S. ruminantium* can contribute to both lactic acid production and utilization. *Selenomonas ruminantium* ssp. *ruminantium* is the more dominant organism (22 to 51% of the total culturable bacteria in the rumens of grain-fed animals; Caldwell and Bryant, 1966), but *S. ruminantium* ssp. *lactilytica* comprises only a small proportion of the total number (Yoshi et al., 2003). *Mitsuokella multiacidus*, a gram-negative rod, is closely related to *S. ruminantium* based on 16S rRNA sequence (Paster et al., 1995). The organism utilizes a similar range of substrates and produces lactic acid as a major fermentation product (Stewart et al., 1997).

Streptococcus bovis is a facultative anaerobe that is normally found in the rumen and in the cecum and colon of cattle. *Streptococcus bovis* counts in forage-fed animals are not high (10^4 to 10^7/g), but its numbers can reach as high as 10^{11}/g of ruminal contents if there is excess fermentable carbohydrate. Although many ruminal bacteria can use starch, the relative success of *Strep. bovis* is because of its rapid growth rate (doubling time as low as 12 min) and rapid degradation of cereal grain starch (McAllister et al., 1990). *Streptococcus bovis* is a mixed acid fermenter (acetate, formate, and ethanol from glucose) but can shift to homolactic (only L-isomer) fermentation if there is excessive substrate and pH is lower than 5.6 (Russell and Hino, 1985; Finlayson, 1986). Although homolactic fermentation produces less ATP (3 mol of ATP/mol of glucose) than mixed acid fermentation (4 mol of ATP/mol of glucose), *Strep. bovis* has a very fast rate of fermentation, and it can

generate more ATP per hour than any other ruminal bacteria (Hungate, 1979). In *Strep. bovis*, pyruvate is converted to lactate by lactic dehydrogenase (**LDH**) or converted to acetyl CoA and formate by pyruvate formate lyase (**PFL**), and the acetyl CoA is then converted to acetate or ethanol. The increase in lactate production at low pH is because the organism allows intracellular pH to decrease to 5.5 when the extracellular pH is below 5.0 (Russell and Hino, 1985; Russell, 1991), and LDH is most active at pH 5.5 (Russell and Hino, 1985). In contrast, the optimal pH of PFL is 7.5 and the activity is less than 10% at pH 6.0 (Asanuma and Hino, 2002a). In addition, activities of both enzymes are affected by the concentrations of allosteric effectors; LDH is affected by fructose-1, 6-diphosphate and PFL by dihydroxy acetone phosphate and glyceraldehyde-3-phosphate (Russell and Hino, 1985; Asanuma et al., 1997, 1999; Asanuma and Hino, 2002a,b). The synthesis of LDH and PFL in *Strep. bovis* is probably regulated at the transcription level (Asanuma and Hino, 2002a).

The explosive growth of *Strep. bovis* in response to availability of fermentable carbohydrate is only observed in situations where the animal is unadapted to grain or during the step-up period. Paradoxically, once cattle are adapted to a grain diet, the numbers of *Strep. bovis* decline 10,000-fold and are similar to those in forage-fed cattle, and the decline is not entirely related to ruminal pH (Wells et al., 1997). Although *Strep. bovis* is considered to be somewhat acid tolerant (Russell, 1991), it is not as tolerant as *Lactobacilli* and its growth rate is reduced if the pH is less than 6.0 (Finlayson, 1986; Wells et al., 1997). Even in an acutely acidotic rumen, the explosive growth of *Strep. bovis* is only transient and numbers decline in concurrence with increases in lactobacilli, suggesting the possibility of a potential antagonism between *Strep. bovis* and *Lactobacilli*. In fact, Wells et al. (1997) identified a bacteriocin produced by *Lactobacillus fermentum*, a species of ruminal lactobacilli that is inhibitory to strains of *Strep. bovis*. In addition, there may be a quorum sensing signal system that allows *Strep. bovis* to monitor its population density. The presence of the *luxS* gene that encodes for an autoinducer-2, an interspecies quorum sensing system, has been demonstrated in *Strep. bovis* (Asanuma et al., 2004). Although *luxS* gene transcription was not directly related to cell density in pure culture, it is conceivable that the autoinducer-2 activity may act as a signal for adjusting cell physiology and metabolism in response to ruminal conditions (Asanuma et al., 2004).

Lactic acid production by *Strep. bovis* causes ruminal pH to decline, which inhibits growth rates of most ruminal bacteria, and the acid-tolerant *Lactobacilli* become predominant. The role of *Strep. bovis* is to initiate the chain of events that will eventually lead to acute ruminal acidosis. Therefore, *Strep. bovis* is considered the major etiologic agent of acute acidosis, and intervention strategies, such as antibiotics and vaccines, are often targeted at controlling the growth of *Strep. bovis* in the rumen (Nagaraja and Miller, 1989; Gill et al., 2000).

Ruminal lactobacilli are more resistant to low pH than *Strep. bovis*, which explains why they become dominant in the acidotic rumen (pH <5.6). A significant increase in the population of ruminal lactobacilli is a common feature of both acute and subacute acidosis (Slyter, 1976; Nagaraja and Miller, 1989; Goad et al., 1998). The rumen has both homofermentative (both L- and D-isomers) and heterofermentative (lactate and acetate or ethanol) lactobacilli, and because there are numerous species, isolates from the rumen are not usually identified at the species level, but rather are often described as "*Lactobacillus* sp." In an early study, 117 strains of lactobacilli were isolated from acid digesta of clinical cases of cattle and sheep, and the species encountered were *L. brevis* (40 strains), *L. bifidus* (45 strains), *L. fermenti* (22 strains), and *L. buchneri* (10 strains) (Krogh, 1963b). Two predominant species of lactobacilli that have been identified and well characterized, particularly in grain-adapted animals, include *L. ruminis* and *L. vitulinus* (Sharpe et al., 1973; Al Jassim and Rowe, 1999). The former species produces primarily L(+) lactic and the latter produces only the D-isomer.

Lactic Acid-Utilizing Bacteria. Lactate is an intermediate product of ruminal fermentation and is further metabolized to VFA. The adaptation of the rumen to the high-grain diet principally involves increases in the populations of bacteria capable of utilizing lactic acid (Huber et al., 1976; Counotte and Prins, 1981). Ruminal bacterial species that ferment lactic acid include *Anaerovibrio lipolytica*, *Fusobacterium necrophorum*, *Megasphaera elsdenii*, *Peptostreptococcus asaccharolyticus*, *S. ruminantium* ssp. *lactilytica*, *Propionibacterium acnes*, and *Veillonella parvula*. Of these, *M. elsdenii* and *S. ruminantium* ssp. *lactilytica* are the predominant lactate-fermenting organisms in grain-fed animals (Huber et al., 1976; Mackie et al., 1978).

Megasphaera elsdenii, a gram-negative and large coccus, is probably the most important ruminal organism with regard to lactic acid fermentation and, therefore, has a central role in the prevention of ruminal lactic acid accumulation in grain-adapted animals (Counotte et al., 1981). *Megasphaera elsdenii* does not utilize starch but can use maltose and glucose and, therefore, is dependent on the amylolytic activities of other bacteria to obtain these energy substrates (Marounek et al., 1989). It is estimated that *M. elsdenii* ferments 60 to 80% of the DL-lactate in the rumen (Counotte et al.,

1981). The reason for such a major role is possibly because *M. elsdenii* is somewhat acid-tolerant (Therion et al., 1982) and its lactate fermentation is not subject to catabolite repression by glucose or maltose (Russell and Baldwin, 1978; Hino et al., 1994). In pure culture studies, the organism does not utilize glucose until the lactate is exhausted (Hino et al., 1994). However, not all strains of *M. elsdenii* exhibit catabolite repression (Marounek et al., 1989). It is interesting that some strains use lactate preferentially to glucose, because lactate yields much less ATP than glucose. Moreover, growth rates of *M. elsdenii* on lactate or glucose do not differ greatly, suggesting that lactate is fermented 5 to 6 times faster than glucose (Hino and Kuroda, 1993; Hino et al., 1994). The organism metabolizes lactate to mainly acetate, propionate, and butyrate and to some extent caproate and valerate (Marounek et al., 1989). It is the only known rumen organism that ferments DL-lactic acid to propionic acid via the acrylate intermediate, but it does not grow on acrylate (Hino and Kuroda, 1993) because acrylate is metabolized exclusively to propionate and no ATP is generated. L-Lactate is converted to propionate via acrylate, and D-lactate is converted to pyruvate by an NAD-independent D-LDH or to L-lactate by lactate racemase, a key enzyme in lactate metabolism (Hino and Kuroda, 1993). Pyruvate produced from D-lactate is then metabolized to acetate, butyrate, valerate, or caproate, producing ATP. The molar proportions of the fermentation products from lactate are influenced by the presence of glucose and differ among strains (Marounek et al., 1989). In the presence of glucose, there is an increase in the production of butyrate, caproate, and valerate with a concurrent reduction in propionate (Marounek et al., 1989). Possibly, propionyl CoA is diverted toward the production of valerate, and products such as butyrate and valerate allow electrons from lactate oxidation to be used. In animals with acidosis experimentally induced with an intraruminal challenge of starch, if lactic acid accumulation is prevented with the use of antibiotics like monensin, large increases in butyrate and valerate concentrations, indicative of *M. elsdenii* activity, have been observed (Nagaraja et al., 1985; Coe et al., 1999). Interestingly, *M. elsdenii* produces propionate from lactate but not from glucose (Marounek et al., 1989); the primary reason appears to be the repression of lactate racemase, induced by lactate, by glucose (Hino and Kuroda, 1993).

Although *S. ruminantium* strains can tolerate low pH, *lactilytica* strains have slow growth rates on lactate under acidic conditions (Therion et al., 1982). In addition, the lactate fermentation by *S. ruminantium* ssp. *lactilytica* is repressed by sugars (Russell and Baldwin, 1978). *Selenomonas ruminantium* ssp. *lactilytica* metabolizes lactate to mainly succinate and propionate. *Selenomonas ruminantium* is less active as a lactate utilizer than *M. elsdenii* because the LDH is suppressed by glucose in *S. ruminantium* (Asanuma and Hino, 2005), but not in *M. elsdenii* (Hino and Kuroda, 1993).

Not much is known about the other lactate-fermenting bacteria, *A. lipolytica*, *F. necrophorum*, *P. acnes*, and *V. parvula*, with regard to their ruminal concentrations and contribution to lactic acid fermentation in the rumen. *Anaerovibrio lipolytica* is a lipolytic and glycerol-fermenting organism, is generally associated with forage feeding (Prins et al., 1975) and the transition from forage to concentrate diets (Slyter et al., 1976), and is not likely to have a significant role in lactate fermentation in grain-adapted animals. *Veillonella parvula*, a gram-negative small coccus, is closely related to *Selenomonas* based on 16S rRNA sequence (Paster et al., 1995). The organism ferments lactate to acetate and propionate and does not ferment any sugars. Gutierrez (1953) isolated *P. acnes* in high numbers from ruminal contents on a lactate medium. Mackie and Gilchrist (1979) reported that *Anaerovibrio* and *Propionibacterium* were the predominant lactate-utilizing bacteria in sheep adapted to a high-grain diet. Huber et al. (1976) identified *M. elsdenii*, *P. asaccharolyticus*, and *S. ruminantium* as the predominant lactic acid-utilizing bacteria in a mature steer adapted stepwise to a high-grain diet.

Fusobacterium necrophorum has garnered considerable attention because of its importance as the primary cause of liver abscesses in cattle (Nagaraja and Chengappa, 1998). The concentration of *F. necrophorum* in forage-fed cattle is in the range of 10^2 to 10^5/g of ruminal contents and the numbers increase to 10^6 to 10^7/g when a grain-based diet is fed (Tan et al., 1994; Coe et al., 1999). Because *F. necrophorum* uses lactate as the major energy substrate and does not use any sugars, the increased counts in grain-fed cattle are reflective of lactate availability. It is well accepted that ruminal lesions from acidosis are the predisposing factors for liver abscesses (Nagaraja and Chengappa,1998). Interestingly, in the acidotic rumen (pH <5.6) the concentration of *F. necrophorum* is reduced or even undetectable (Coe et al., 1999). The reduction is probably pH-mediated because the optimal pH for *F. necrophorum* growth is 7.4 and a pH of 5.6 or below is inhibitory (Tan et al., 1996). Acidosis is a predisposing factor for liver abscesses and although ruminal acidity may kill *Fusobacterium* in ruminal contents, it may not have any effect on *Fusobacterium* associated with the ruminal wall (Narayanan et al., 1997).

Ruminal Acidosis and Ciliated Protozoal Changes

Ruminal ciliated protozoa are similar to bacteria in that their numbers increase in response to increased

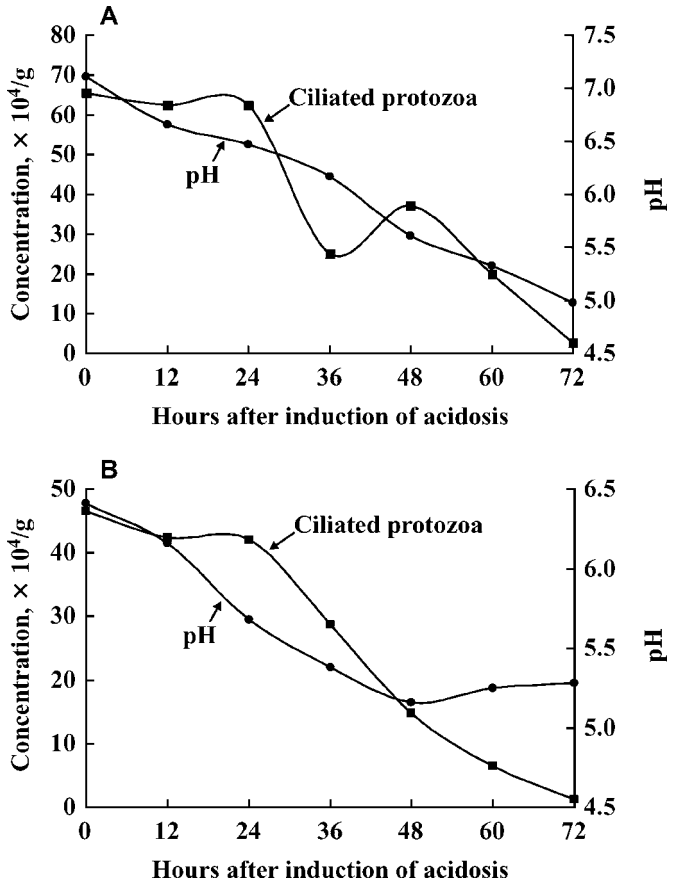

Figure 4. Ruminal ciliated protozoal population (■) and ruminal pH (●) in cattle with experimentally induced acute acidosis (A; data from Nagaraja and Towne, 1990) and subacute acidosis (B; data from Goad et al., 1998).

substrate availability. Moderate increases in grain intake result in large increases in the numbers of ciliated protozoa (Dennis et al., 1983). In contrast, high- or all-grain diets have variable effects, including total elimination of protozoa in some animals (Vance et al., 1972; Towne et al., 1990a,b; Franzolin and Dehority, 1996). Ciliated protozoa are believed to be much more sensitive than bacteria to fluctuations and reductions in ruminal pH, hence, ruminal pH is a critical factor in the maintenance of ciliated protozoa in the rumen. In fact, reduction in ruminal pH is a method for experimental defaunation in ruminants (Whitelaw et al., 1984). Therefore, it is not surprising that a marked reduction in numbers of ciliated protozoa is a consistent microbial change associated with ruminal acidosis (Nagaraja and Towne, 1990; Braun et al., 1992; Goad et al., 1998). The significant reduction in protozoal numbers, and in some instances a complete elimination, is a common feature of both acute and subacute acidosis (Figure 4). Ciliated protozoal genera seemingly resistant to lower pH include *Entodinium*, *Polyplastron*, *Isotricha*, and *Dasytricha* (Towne et al., 1990a). The genus *Entodinium* is the ruminal protozoan most resistant to low pH, which explains why the genus is the most dominant, as much as 90 to 99% of the total population, in cattle fed high-grain diets (Towne et al., 1990a,b; Franzolin and Dehority, 1996; Hristov et al., 2001).

Ciliated protozoa have a significant role in ruminal starch and lactic acid metabolism (Nagaraja and Towne, 1990). In terms of lactic acid production, the holotrichid protozoa *Isotricha* and *Dasytricha* produce both isomers of lactic acid as a major product of starch or sugar fermentation, whereas entodiniomorphs produce only trace amounts (Bonhomme, 1990). On the other hand, lactic acid fermentation is only associated with entodiniomorphs and not with holotrich protozoa (Newbold et al., 1987). Although the relative contribution of ciliated protozoa to ruminal lactate pool size and turnover is not known, there is generally an inverse relationship between ciliated protozoa and lactic acid concentration in the rumen (Nagaraja et al., 1986; Newbold et al., 1986). This is likely due to ciliated protozoal uptake of sugars and starch, which sequesters them from bacterial fermentation. Holotrichid protozoa assimilate sugars and store them as amylopectin, which is then metabolized slowly to produce VFA. Entodiniomorphid ciliates use little or no sugars, but actively ingest starch granules, which are converted to a storage form and metabolized slowly. Moreover, there is enhanced lactate clearance in the presence of entodiniomorphid protozoa (Newbold et al., 1986, 1987). More importantly, ciliated protozoa may have an indirect influence on lactic acid production or accumulation because of their interaction with bacteria. Primarily because of their predatory activity, the presence of ciliated protozoa is associated with reduced bacterial density in the rumen (Bonhomme, 1990). Therefore, ciliated protozoa are capable of reducing the rate and extent of starch fermentation in the rumen (Mendoza et al., 1993). The slowing of starch fermentation should result in more stable VFA production and higher postprandial pH in the rumen. The reduction in bacterial activity accounts for the moderating effect on ruminal fermentation that ciliated protozoa exert in animals fed high-grain diets. This is supported by higher pH values and lower VFA concentrations in rumens of cattle that have normal protozoal concentrations compared with those that were defaunated (Veira, 1986; Nagaraja et al., 1992). Therefore, ciliated protozoa have a beneficial role, termed "buffering effect" by Hungate (1978), in the rumens of grain-fed cattle. This is contrary to the perception that the contribution of ciliated protozoa to the ruminal metabolism of high-grain fed cattle is not significant because starch-based diets reduce or even

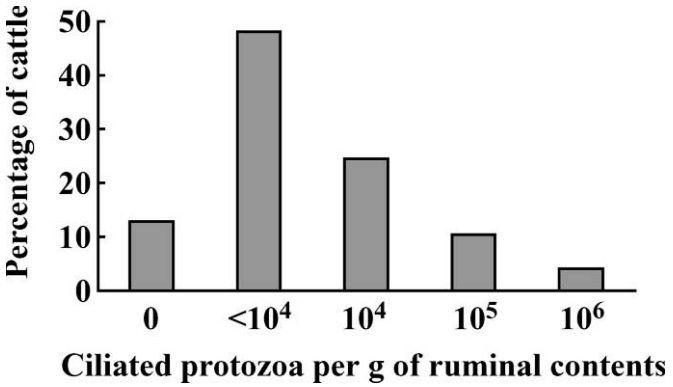

Figure 5. Distribution of numbers of ruminal ciliated protozoa in feedlot cattle. Ruminal samples were collected at slaughter from 364 cattle fed different finishing diets (data from Towne et al., 1990b).

eliminate protozoal populations (Hungate, 1978). The reduction or elimination is usually attributed to fluctuating and relatively low ruminal pH, faster passage rates, smaller feed particles, and hypertonicity associated with grain feeding (Nagaraja and Towne, 1990). Studies have shown that rumens of grain-fed cattle harbor a resilient but volatile population of ciliated protozoal population (Figure 5) and that fluctuation is in response to the dynamic ruminal conditions (Towne et al., 1990a; Franzolin and Dehority, 1996; Hristov et al., 2001). In addition to the volatility of the population, there is considerable reduction in genetic diversity of ciliated protozoal population in grain-fed cattle (Towne et al., 1990a). A certain fraction of feedlot cattle is defaunated (10 to 15%) at any given time, but defaunation is transient and ciliated protozoa reappear when the ruminal conditions become hospitable (Towne et al., 1990a). The source for refaunation may be a faunated cohort in the pen or endogenous, with the ciliated protozoa either surviving in the rumen at undetectable levels or emigrating from the omasum (Towne and Nagaraja, 1990a).

Ruminal Acidosis and Microbial Toxic Products

Although ruminal acids are considered the main contributors to the pathophysiology of acidosis, other toxic factors of microbial origin have been implicated to play a role (Dunlop, 1972; Owens et al., 1998). Other compounds considered as possible toxic factors are ethanol, amines, bacterial endotoxins, and possibly other unidentified toxins. The ruminal concentration of ethanol (mainly a product of heterofermentative lactobacilli) increases under acidotic conditions (Allison et al., 1964b), but it is not high enough to be of any significance. Furthermore, both the microbes in the rumen and the animal are capable of metabolizing ethanol. Amines and bacterial endotoxins are believed to have a role in the pathogenesis of acidosis.

Amines. Pharmacologically active amines, such as histamine, tyramine, and tryptamine are produced in the rumen by decarboxylation of precursor AA. Among the 3 amines, histamine has received considerable attention because of its putative role in laminitis. Destruction of the normal hemodynamic process is a major factor in the development of laminitis (Nocek, 1997). Histamine is a potent vasodilator and increases capillary permeability (Brent, 1976), and the association of histamine fits well with the nutritional theory of laminitis development (Nocek, 1997). The ruminal production and accumulation of histamine are generally associated with low pH (Dain et al., 1955; Van Der Horst, 1961; Irwin et al., 1979). Amino acid decarboxylases are inducible intracellular enzymes and their induction by AA generally occurs at acidic pH (Morris and Fillingame, 1974). Therefore, production of the highly basic amines in the acidotic rumen constitutes an effort to regulate pH. Irwin et al. (1979) measured ruminal concentrations of histamine, tyramine, and tryptamine in sheep following dosing via stomach tube a mixture of 90% glucose and 10% casein. Ruminal tyramine and tryptamine concentrations increased with decreased ruminal pH, but histamine concentration did not change significantly. Others have shown a direct relationship between ruminal pH and histamine concentration (Dain et al., 1955; Wilson et al., 1975). Not much is known about the production and fate of tyramine and tryptamine in the rumen. It is generally accepted that the source of decarboxylases is the acid-tolerant bacteria in the rumen, although decarboxylases might be activated by low pH. Histamine-producing bacteria are undetectable or present in extremely low concentrations in forage-fed cattle and numbers up to 10^7/g are present in grain-fed cattle (Garner et al., 2002). Initially, ruminal lactobacilli were considered the main producers of histidine decarboxylase (Rodwell, 1953). Recent research of Russell and his associates has identified a previously unrecognized species, *Allisonella histaminiformans* (named in honor of Milton J. Allison, a rumen microbiologist), a gram-negative and ovoid species, as an important producer of histamine in the rumen (Garner et al., 2002). The organism is highly specialized in that it catabolizes histidine as its sole energy source and produces histamine in a 1:1 ratio of histidine fermentation. The organism is acid tolerant and is capable of initiating growth even at pH 4.5 (Garner et al., 2002). Interestingly, the organism was readily isolated from silage-fed animals but not from hay-fed animals, and it was suggested that the organism requires peptide-N, which would be readily supplied by silage, for growth (Garner et al., 2004).

Because histamine is a potent hemodynamic effector and disturbance of normal hemodynamic process is a major etiological factor of laminitis, it has long been suspected that ruminal histamine is related to the onset of laminitis (Nocek, 1997). Ruminal concentration of histamine is highly variable (Koers et al., 1976), and high concentrations have been noted even under nonacidotic conditions of the rumen (Sjaastad, 1967a). However, whether ruminal histamine is absorbed or not has been a subject of controversy (Sjaastad, 1967b; Brent, 1976; Braun et al., 1992). Some contend that ruminal histamine is not absorbed from the rumen and plasma histamine concentration originates from tissue release (Suber et al., 1979). A study (Aschenbach and Gabel, 2000) that used Ussing chambers indicated significant flux of histamine in ruminal epithelium exposed to acidity (pH 5.1). The increase in absorption may be because of increased epithelial permeability or decreased histamine catabolism in the epithelial cells. There is evidence that a pH-induced decline in histamine catabolism by diamine oxidase activity in the ruminal epithelium is the likely reason (Dickinson and Huber, 1972).

Endotoxins. Endotoxin or lipopolysaccharide (LPS) is a cell wall component of all gram-negative bacteria, regardless of their pathogenicity. Mixed ruminal bacteria have been shown to contain endotoxic LPS (Nagaraja et al., 1978b, 1979b). Among ruminal bacterial species, *M. elsdenii* and *S. ruminantium* contain LPS (Nagaraja et al., 1979c; Takatsuka and Kamio, 2004) and, in case of *M. elsdenii*, the LPS possesses the characteristic biological activity, although the potency is considerably lower than that of the classical LPS of *Escherichia coli*. However, *Fibrobacter succinogenes*, a cellulolytic organism typically found in the rumen of forage-fed cattle, lacks cell wall LPS (Vinogradov et al., 2001). Because ruminal bacteria are predominantly gram-negative and death and disintegration of a certain number of bacteria in a population are normal bacterial processes, it is not surprising that endotoxin is normally present in ruminal fluid. Initial studies on detection and quantification of endotoxin were based on biological assays, such as mouse lethality with actinomycin D, an endotoxin-potentiating agent (Mullenax et al., 1966; Nagaraja et al., 1978c). Subsequently, a more specific and sensitive method, the limulus amoebocyte lysate test, based on the ability of endotoxin to cause gelation of lysates derived from the blood cells (amoebocytes) of the horseshoe crab (*Limulus polyphemus*), has been used (Andersen et al., 1994a; Gozho et al., 2005). Interestingly, the concentration of endotoxin is higher in grain-fed compared with forage-fed cattle (Nagaraja et al., 1978c; Andersen et al., 1994a). The higher concentration of endotoxin may be because of higher numbers of gram-negative bacteria or perhaps conditions in the rumen of grain-fed animals (e.g., lower ruminal pH, higher osmolality) are favorable for the release of endotoxin from intact bacteria (Nagaraja et al., 1978c).

Ruminal endotoxins have long been suspected to contribute to the pathogenesis of ruminal acidosis (Dougherty, 1976; Huber, 1976). The clinical and blood biochemical changes associated with ruminal acidosis are somewhat similar to those observed following endotoxin administration (Nagaraja et al., 1979a; Aiumlamai et al., 1992; Jacobsen et al., 2005). In the acidotic rumen, either because of precipitous drop (as in acute acidosis) or wide fluctuations in ruminal pH (as in subacute acidosis), gram-negative bacteria could be lysed, thus releasing significant concentrations of free endotoxins in the rumen (Table 2). Dougherty and associates demonstrated than an unidentified toxic substance in ruminal fluid and plasma of overfed sheep, when administered intravenously to dogs and sheep, evoked physiological responses (leukopenia, blood pressure changes) similar to that of endotoxic LPS (Dougherty and Cello, 1949; Mullenax et al., 1966). An increase in the concentration of free endotoxin in the rumen following experimental induction of acute (Nagaraja et al., 1978a; Andersen and Jarløv, 1990; Aiumlamai et al., 1992; Andersen et al., 1994b) or subacute acidosis (Gozho et al., 2005) has been demonstrated. There are reports of endotoxin presence in peripheral blood of cattle with experimentally induced acidosis (Dougherty et al., 1975a; Aiumlamai et al., 1992), and release of inflammatory mediators, such as arachidonic acid metabolites and cytokines (IL and tumor necrosis factor) following induction of ruminal acidosis (Andersen and Jarløv, 1990; Aiumlamai et al., 1992; Andersen et al., 1994b). However, not all studies have succeeded in demonstrating endotoxemia (Andersen and Jarløv, 1990; Table 3). Another biological effect of endotoxin that is of relevance to acidosis is the inhibition of reticuloruminal motility associated with endotoxemia (Eades, 1997). Inhibition of reticuloruminal motility could have the benefit of reducing the fermentation rate because of reduced mixing, but at the same time, it could exacerbate ruminal acidosis by reducing the rate of absorption or passage of acids from the rumen.

For rumen bacterial endotoxin to participate in the pathogenesis of acidosis, it should be absorbed into the blood from the gastrointestinal tract. Whether endotoxin can be absorbed or translocated from the rumen or elsewhere in the gut into the blood remains unresolved. Because of the large pool of endotoxin and the availability of a large absorptive surface, the rumen appears to be the logical site for endotoxin uptake. Moreover, endotoxin passed into the abomasum or small intestine may be inactivated by acid or enzymes (Nagaraja et al., 1979a). Huber et al. (1979) using leucopenia as the

Table 2. Bacterial endotoxins in ruminal fluid or blood of cattle or sheep experiencing acidosis

Reference	Type of acidosis and experimental animal	Methodology	Endotoxin in ruminal fluid	Endotoxin in blood	Inflammatory indicators in blood
Dougherty and Cello, 1949	Acute acidosis in sheep	Physiological and biochemical observations in anesthetized dogs injected with ruminal fluid or heparinized blood	Present	Present	Fall in blood pressure
Mullenax et al., 1966	Nonacidotic cattle	Physiological and hematological responses following intravenous injection of rumen bacterial extract in cattle and sheep	Present	Not determined	Leukopenia followed by leukocytosis
Dougherty et al., 1975a	Acute acidosis in sheep and cattle	Limulus amoebocyte lysate (LAL) test	Not determined	Present	Not determined
Nagaraja et al., 1978a	Acute acidosis in cattle	Mouse lethality test using endotoxin potentiating agent (actinomycin D)	Present	Negative	Increased blood neutrophils
Andersen and Jarløv, 1990	Acute acidosis in cows	LAL test and measurement of inflammatory mediators in blood	Present	Negative	Nonsignificant elevation in arachidonic acid metabolites (prostaglandins)
Aiumlamai et al., 1992	Acute acidosis in calves	LAL test and measurement of inflammatory mediators in blood	Not determined	Present	Increased cytokines and arachidonic acid metabolites (prostaglandins)
Andersen et al., 1994b	Acute acidosis in cows	LAL test and measurement of inflammatory mediators in blood	Not determined	Present	Increased arachidonic acid metabolites (prostaglandins)
Gozho et al., 2005	Subacute acidosis in steers	LAL test and measurement of inflammatory mediators in blood	Increased	Not determined	Increased levels of haptoglobin and serum amyloid-A

criterion were unable to demonstrate endotoxin absorption from the duodenum of either normal or lactic acidotic sheep. We have studied ruminal absorption of endotoxin in steers by administering ^{51}Cr-labeled *E. coli* endotoxin into the rumen (Lassman, 1980; Anderson, 1984). None of the steers, whether forage-fed (100% alfalfa hay diet), grain-fed (92% concentrate diet based on sorghum grain), or ruminally acidotic, showed evidence of absorption either through lymph (thoracic duct) or blood (portal vein), and it was concluded that the ruminal epithelium is impermeable to endotoxin.

ACIDOSIS RESEARCH MODELS

Most of the information on ruminal and systemic changes associated with acidosis has been derived from experimentally induced acidosis in cattle and sheep. A variety of methods have been used, generally involving ruminally cannulated animals either dosed intraruminally with fermentable carbohydrates or allowed to consume excessive amounts of a grain diet (Table 3). Such approaches usually succeed in inducing ruminal acidosis and allow close monitoring of ruminal pH to allow termination of the experiment before systemic acidosis becomes irreversible.

Experimentally Induced Acute Acidosis

Acute ruminal acidosis is typically induced experimentally by placing a large quantity of readily fermentable carbohydrates in the rumen of cattle, particularly of those that have not been adapted to a grain-based diet. Various challenge models have led to acute acidosis in cattle (Table 3), including 7% of BW of a mixture of 75% ground corn and 25% ground oats dosed ruminally to cattle adapted to alfalfa (Dougherty et al., 1975b); 3% of BW as steam-flaked corn dosed ruminally to steers adapted to grass hay (Hibbard et al., 1995; Brown et al., 2000); 2.75% of BW of ground corn dosed ruminally to steers adapted to 80% alfalfa:20% grain (Nagaraja et al., 1981); 1.25% of BW as glucose dosed ruminally to cattle adapted to alfalfa (Harmon et al., 1985) or 80% alfalfa:20% grain (Nagaraja et al., 1982); 1.25% of BW as a ground corn:corn starch mixture dosed ruminally each day (typically requiring 2 doses) to steers adapted to 80% alfalfa:20% grain until an acute acidosis was induced (Nagaraja et al., 1985; Coe et al., 1999). For these models, cattle are typically fasted before the carbohydrate loading to ensure adequate space within the rumen for the substrate. In most cases, cattle are adapted to forage so that lactate-utilizing organ-

Table 3. Acidosis challenge models with beef cattle

Reference	Adaptation diet	Challenge[1]	Minimum ruminal pH[2]	Peak ruminal lactate[2] (m*M*)	Intervention[3]
Acute acidosis					
Dougherty et al., 1975b	Alfalfa	IR; 7% BW of 75% corn:25% oats	4.0	NM	2 of 3 died
Nagaraja et al., 1981	Alfalfa	IR; 1.25% BW of glucose	4.2	58	12 h or pH <4.3
Nagaraja et al., 1981	Alfalfa	IR; 2.75% BW of ground corn	4.3	140	48 h or pH <4.3
Nagaraja et al., 1982	80% alfalfa: 20% grain	IR; 1.25% BW of glucose	4.2	71	12 h
Harmon et al., 1985	Alfalfa	IR; 1.2% BW of glucose	4.2	120	48 h
Nagaraja et al., 1985; Coe et al., 1999	80% alfalfa: 20% grain	IR; 1.25% BW of corn:corn starch, repeated daily	4.4	75	pH <4.5
Hibbard et al., 1995	Grass hay	IR; 3% BW of steam-flaked corn	4.8	112	12 h
Brown et al., 2000	Grass hay	IR; 3% BW of steam-flaked corn	Ave 5.0	Ave 37	14 d; 2 of 5 removed from trial
Subacute acidosis					
Harmon et al., 1985	Alfalfa	DS to 70% concentrate (ad libitum)	5.5	<10	48 h
Hibbard et al., 1995	50% concentrate	IR; 1.5% BW of corn:wheat	5.6	NM	24 h
Goad et al., 1998	80% alfalfa: 20% grain	DS to all-grain diet, 1.75 × maintenance every 12 h	5.3	<5	72 h
Goad et al., 1998	20% alfalfa: 80% grain	DS to all-grain diet, 1.75 × maintenance every 12 h	5.2	<5	72 h
Montano et al., 1999	90% concentrate	IR; 0.33% BW of glucose	5.1	22	7 h
Brown et al., 2000	50% concentrate	IR; 1.5% BW of corn:wheat	Ave 5.7	Ave 17	14 d
Brown et al., 2000	Grass hay	DS to 50% concentrate (ad libitum)	Ave 5.8	Ave 48	14 d

[1]IR = intraruminal dose; DS = diet switch.

[2]Ave = daily average; NM = not measured.

[3]Predetermined time for end of trial or ruminal pH at which rumen was evacuated.

isms in the rumen are at low concentrations, although inclusion of a small amount of grain (10 to 20%) in the adaptation diet may better induce ruminal acidosis by ensuring that adequate numbers of amylolytic organisms are present to ferment the dosed carbohydrates (unpublished observations of T. G. Nagaraja).

With these models of acute ruminal acidosis, lactate accumulation occurs in the rumen, often exceeding 100 m*M* at peak (Nagaraja et al., 1981; Harmon et al., 1985; Hibbard et al., 1995), which leads to ruminal pH less than 5.0 and often near 4.0. The experiment is often terminated when ruminal pH reaches a threshold of 4.2 to 4.5, with the ruminal contents being removed and replaced with contents of a forage-fed animal (Nagaraja et al., 1981, 1985). In cases where intervention has not been practiced (e.g., to test the long-term effect of acidosis), death or euthanasia of the animal is a common outcome (Dougherty et al., 1975b; Brown et al., 2000). In some cases, the acidosis is terminated based on a predetermined time end-point (e.g., 12 h; Hibbard et al., 1995) by removing ruminal contents.

In association with the depression in ruminal pH and the accumulation in ruminal lactate, the typical acidosis-related shifts in microbial populations are observed with flourishing lactate producers and reductions in populations of lactate utilizers and of protozoa. Effects of systemic acidosis are also observed with elevations of blood lactate (Harmon et al., 1985; Brown et al., 2000) and decreases in blood pH, base excess, and bicarbonate, and increases in packed cell volume (Dougherty et al., 1975b; Nagaraja et al., 1981, 1982, 1985).

In these challenge models, long-term effects of acidosis are likely if intervention strategies are not utilized, with feed intake greatly reduced for an extended period (Brown et al., 2000). Even in situations where intervention strategies have been utilized, there may be long-term effects on the animals. Krehbiel et al. (1995a) challenged lambs adapted to a 50% concentrate diet with 1.8% of BW as a single ruminal dose of glucose. Within 16 h of dosing, 3 of 5 lambs reached a ruminal pH of 4.2 or less and subsequently had their ruminal contents evacuated and replaced with ruminal contents from a donor steer fed alfalfa. At 10 d after the challenge, liquid passage rates from the rumen were numerically lower for challenged than for control lambs (16.7 vs. 26.9%/h), and this trend continued for lambs at 3 mo after the challenge (18.1 vs. 26.3%/h) and became significant at 6 mo after the challenge (14.7 vs. 32.8%/h). Although rates of ruminal absorption of VFA were not markedly affected at 10 d after the acidosis challenge, those lambs challenged with glucose demonstrated numerically lower rates of absorption of acetate, propionate, and butyrate at 3 and 6 mo after the acidosis

event (significant for propionate absorption at 6 mo). These results from sheep suggest that the use of animals for repeated acidosis challenges could lead to altered responses to treatments.

Relatively large variability in the response of animals to acute acidosis challenges can also be problematic for researchers. The challenge used by Dougherty et al. (1975b; 7% of BW as ground grain) resulted in death of 2 of the 3 cattle at 48 and 76 h after the challenge; these cattle experienced a low ruminal pH (near 4) that would be associated with ruminal lactate accumulation. In contrast, 1 steer did not develop a significant ruminal acidosis (pH nadir = 5.6) and demonstrated few symptoms except severe diarrhea. Brown et al. (2000) also observed large variation in response of individual animals to their acidosis challenge. Of the 5 steers dosed with steam-flaked corn at 3% of BW, 3 developed acute acidosis (average ruminal pH less than 5.0 on the challenge day), 1 developed only a subacute acidosis (average ruminal pH less than 5.6 on the challenge day), and 1 did not develop ruminal acidosis. It is clear that this type of variation leads to the need for large numbers of animals when these challenge models are used to evaluate the effect of treatments on ruminal acidosis.

Experimentally Induced Subacute Acidosis

When smaller amounts of carbohydrates are used in an acidosis challenge, it is possible to develop a subacute rather than an acute acidosis. Under these conditions, there is little lactate accumulation in the rumen, the drop in ruminal pH is due to increased concentrations of VFA (Table 3), ruminal pH typically does not decline below 5.0, and animals often will exhibit a normally functioning rumen within 1 d. The increase in ruminal VFA without accumulation of lactic acid is indicative of lactate-utilizing organisms keeping pace with lactate production, such that major shifts in pH, and subsequently in microbial populations, do not occur. In subacute acidosis models, fermentable substrate is directly dosed to the rumen through a cannula or the animal is provided a diet rich in fermentable carbohydrates. Cattle are often fasted before the challenge to stimulate rapid and large consumption of the acidosis-inducing diet.

Because subacute acidosis challenges are not designed to cause accumulation of lactate in the rumen, it is not necessary for cattle to be adapted to a forage-based diet before the challenge. For example, Montaño et al. (1999) utilized cattle that were adapted to a 90% concentrate diet and challenged them with glucose dosed ruminally at 0.33% of BW. This challenge led to short-term (<7 h) decreases in ruminal pH (5.1 at 3 h after the challenge) and only moderate and transient increases in ruminal lactate (peak near 22 m*M* at 3 h after the challenge). Ruminal VFA concentrations were increased.

Harmon et al. (1985) induced subacute acidosis in steers by switching cattle immediately from a limit-fed (1.5% of BW daily) diet of alfalfa hay to a 70% concentrate diet. On the first day of the 70% concentrate diet, steers consumed about 3.9% of BW, whereas intake dropped to about 0.5% of BW on the second day. Despite the large amount of fermentable carbohydrates consumed by the steers on the first day of the challenge, ruminal pH did not drop below 5.5 and lactate did not accumulate in the rumen. Blood pH and bicarbonate both decreased following the diet change, indicating that systemic acidosis was induced. The amount of carbohydrate consumed (3.9% of BW of a 70% concentrate diet providing 2.7% of BW as concentrate) by steers in the study of Harmon et al. (1985) would be expected to induce an acute acidosis if provided directly to the rumen of the cattle.

Goad et al. (1998) compared ruminal changes following induction of ruminal acidosis in cattle adapted to a high-hay or high-grain diet. The 2 diets resulted in different proportions of lactate-producing and lactate-utilizing organisms in the rumen. The challenge involved withholding feed for 24 h followed by overfeeding an all-grain diet at twice the previous daily intake. In theory, cattle not adapted to grain should exhibit greater susceptibility to subacute acidosis because of lower numbers of lactate-utilizing bacteria and higher numbers of ciliated protozoa. However, ruminal fermentation patterns associated with subacute acidosis were similar following overfeeding regardless of whether steers were adapted to a grain- or forage-based diet (Goad et al., 1998).

In the studies of Harmon et al. (1985) and Goad et al. (1998), the development of subacute acidosis rather than of acute acidosis may be a result of slow intake of the diet when consumed by the cattle, the buffering of saliva produced during consumption of the diet, or the dilution (buffering) provided by the forage portion of the diet. Regardless of the cause, the induction of acidosis appears to follow a different course when a carbohydrate challenge is ruminally dosed than when it is consumed by the animal.

Steers challenged with 1.25% of BW of a mixture of ground corn and corn starch did not develop acute acidosis with ruminal lactate accumulation following a single dose administered to steers adapted to an 80% alfalfa:20% grain diet; however, following a second dose (24 h after the initial dose), large concentrations of ruminal lactate appeared and ruminal pH was reduced below 4.5 (Nagaraja et al., 1985; Coe et al., 1999). Krehbiel et al. (1995b) dosed cattle adapted to 70% concen-

trate diets with dry-rolled corn at 1.3% of BW and observed that ruminal pH was reduced below 5.2 (nadir at 15 h after the challenge). Lactate accumulation was transient and returned to baseline amounts within 1 d. For cattle adapted to a 50% concentrate diet, a ruminal challenge of 1.5% of BW as a wheat-corn mixture dropped ruminal pH from 6.7 to 5.6 (Hibbard et al., 1995), but did not lead to much ruminal accumulation of lactic acid (Brown et al., 2000). Taken as a whole, these studies indicate that single doses of a grain-based challenge up to 1.5% of BW will cause a subacute ruminal acidosis, without great risk of developing an acute ruminal acidosis.

Amounts of glucose required to induce subacute acidosis are significantly less than amounts of grain. Glucose at 1.2% of BW dosed ruminally led to acute acidosis (Nagaraja et al., 1982; Harmon et al., 1985) in cattle adapted to alfalfa or alfalfa-based diets, whereas a much lower dose (0.33% of BW) led to only a subacute acidosis in steers adapted to a grain-based diet (Montaño et al., 1999). In work with sheep adapted to a 50% concentrate diet, Krehbiel et al. (1995a) dosed glucose intraruminally in amounts of 0, 0.6, 1.2, and 1.8% of BW. Glucose at 0.6% of BW led to small, transient increases in ruminal lactate with no effect on ruminal VFA concentrations, although ruminal pH did drop below 5.0 for a short time. In contrast, glucose dosed at 1.2 or 1.8% of BW led to greater and more sustained ruminal lactate concentrations, longer periods when ruminal pH was below 5.0, and reductions in ruminal VFA concentrations, all indicators that an acute acidosis was induced. Thus, it appears that a ruminal dose of 0.6% of BW of glucose was slightly less than needed to induce acute ruminal acidosis in sheep. The data suggest that amounts of glucose that will induce a subacute acidosis without great risk of an acute acidosis are less than half of amounts provided as grain, and amounts between 0.3 and 0.6% of BW are suggested as appropriate.

Effect of Variation in Feed Intake on Ruminal Acidosis

Cooper et al. (1999) studied the impact of daily variations in feed intake of cattle on ruminal pH by feeding either a constant amount or by varying intake daily such that the amount of feed offered varied by as much as 1.8 kg/d. Variations in daily intake of up to 1.8 kg/d led to only small changes in ruminal pH. When steers were limit-fed, average ruminal pH was not affected by daily variation in feed intake, although the area of the ruminal pH curve below 5.6 was increased in 1 of 2 trials. In contrast, when steers were fed at ad libitum levels, average ruminal pH was increased (from 5.55 to 5.72) and the area of the ruminal pH curve below 5.6 was decreased by induced daily variation in the feed intake in 1 of 2 trials. In agreement with the relatively small effects on ruminal pH, imposed variation in intake of 1.8 kg/d did not affect performance of finishing cattle (8 or 9 steers/pen).

Schwartzkopf-Genswein et al. (2004) used 3-d fluctuations in intake (90% of ad libitum intake for 3 d followed by 110% of ad libitum intake for 3 d) to assess the effect of feed intake variation on ruminal acidosis. Similar to the results of Cooper et al. (1999), there were no large differences in average ruminal pH in response to the induced variation in intake. The pattern of ruminal pH over time did suggest that cattle offered a fluctuating amount of feed consumed their feed more quickly, which agrees with numerical differences in rate of feed intake observed by Cooper et al. (1999).

Soto-Navarro et al. (2000) altered feed intake by 10% daily over 4-d cycles (+10%, 0, –10%, 0) and assessed ruminal acidosis in steers. Clear effects of the intake variation were not evident. Cattle fed once daily had lower ruminal pH when intake was variable, whereas cattle fed twice daily had greater ruminal pH when intake was variable. The nadir in pH was lower for cattle fed once daily, suggesting that cattle fed less frequently would be more subject to ruminal acidosis, and, as such, those fed once daily may represent a better model for evaluating the effects of treatments on the risk of ruminal acidosis.

The model utilized by Erickson et al. (2003) to assess the effect of variation in feed intake on ruminal acidosis involved offering cattle 125% of daily intake 4 h after the time at which they were accustomed to being fed. This variation in timing and amount of feed did not strikingly alter ruminal pH or ruminal pH variance, and cattle demonstrated normal intake and ruminal function in the 4 d following the challenge. The model appeared useful for assessing the impact of treatments on feed intake patterns; cattle adapted to clean-bunk management consumed their feed more quickly than those adapted to ad libitum intake, and monensin tended to reduce rate of intake and meal size while increasing the number of meals daily both before and after, but not on, the day of the challenge.

Impact of Diet Changes on Ruminal Acidosis

Cattle often undergo acidotic challenges in the feed yard when they are transitioned from forage-based to concentrate-based diets as part of normal feedlot management. A number of studies have evaluated the impacts of different step-up protocols on ruminal acidosis. In a trial designed to evaluate the effectiveness of antibiotics in reducing ruminal acidosis, Coe et al. (1999)

abruptly switched steers from alfalfa hay to a 70% concentrate diet. After 3 d of the 70% concentrate diet, cattle were switched to an 85% concentrate diet, and 3 d later to a 100% concentrate diet. The intake of the diet was limited to 2.5% of BW daily, and cattle were not fasted before the dietary changes. No lactate accumulated in the rumen during the dietary changes, and ruminal pH averaged 6.20 for the 70% concentrate diet, 5.76 for the 85% concentrate diet, and 5.68 for the 100% concentrate diet. Ruminal VFA concentrations were markedly higher when steers received any of the concentrate diets than when they received alfalfa hay. Responses to the switch from alfalfa to 70% concentrate were generally similar to those of Harmon et al. (1985), although feed intake and ruminal function appeared more stable in the study of Coe et al. (1999), likely due to the limitation in feed intake to 2.5% of BW daily, which would reduce the total carbohydrate load into the rumen each day.

Leedle et al. (1995) tracked the changes in ruminal pH as concentrate proportion was increased weekly in the diet of cows, with diets containing 25, 50, 75, and 90% concentrate. Diets were limit-fed at 2% of BW daily, with feed remaining 2 h after feeding being placed directly in the rumen over a 5-h period. Ruminal pH declined as concentrate in the diet increased, and for each diet pH continued to decrease over the first 5 d that they were offered. Ruminal pH averaged less than 5.4 on the fifth day that the 90% concentrate diet was offered, and 1 of 6 cows developed an acute ruminal lactic acidosis by that time. Intakes of the 90% concentrate diet by the cows were less than one-third of the total offered, which, because orts were placed directly in the rumen, may have resulted in an acute acidosis challenge model similar to that described by Nagaraja et al. (1985) and Coe et al. (1999).

Other researchers have observed that typical and faster-than-typical step-up protocols rarely result in acute acidosis under research conditions. Galyean et al. (1992) switched cattle from 75 to 90% concentrate diets and observed no substantial accumulation of ruminal lactate, with ruminal pH averaging around 5.6. Thus, acute acidosis was not associated with this relatively modest shift in dietary concentrate level. Bevans et al. (2005) considered the impact of rapidly changing cattle from 40 to 90% concentrate diets through use of a single intermediate diet (65% concentrate) fed for 3 d compared with a more traditional step-up protocol that used 5 intermediate diets (48, 57, 65, 73, and 82% concentrate) each being fed for 3 d. Ruminal measures were collected on the first 3 or 4 d that cattle were offered the 65 and 90% concentrate diets. Acute acidosis did not develop with either step-up protocol, and the model was generally unable to detect differences between the 2 protocols in ruminal pH. The inability to detect differences between protocols likely reflects large variation in ruminal pH that occurred consequent to variations in feed intake. This research points out the variability among animals in both DMI and ruminal pH, and thus the need for large numbers of observations to detect treatment differences. The cattle on both step-up protocols demonstrated reductions in DMI on the second day that the 90% concentrate diet was fed, which may have limited the development of ruminal acidosis.

Evaluating Ruminal Acidosis During Digestion Studies

Cattle fed grain-based diets, with no attempt by the researchers to induce acidosis, can serve as a useful model for studying ruminal acidosis. Cattle fed grain-based diets will often exhibit an average ruminal pH of 5.6 to 5.8 with daily nadirs of 5.0 being common (Cooper et al., 1999; Schwartzkopf-Genswein et al., 2004). Thus, a typical feedlot diet fed under typical feedlot conditions could be considered an acidotic challenge. A large number of digestion trials have been conducted with cattle fed grain-based diets, and results are useful for assessing the potential for different diets and management practices to induce acidosis in cattle. Several examples would include higher ruminal pH for steers fed dry-rolled corn than for those fed high-moisture or steam-flaked corn (Cooper et al., 2002); higher ruminal pH for steers fed higher levels of forage (Zinn et al., 1994; Calderon-Cortes and Zinn, 1996); and increases in intake leading to lower ruminal pH (Kreikemeier et al., 1990).

Response Criteria for Acidosis Challenge Studies

In addition to the frequently measured criteria discussed above (ruminal pH, lactate, and VFA; DMI; microbial populations; blood gases), there are several response criteria that could provide valuable information related to ruminal acidosis in beef cattle. One measure of damage resulting from an acidosis event would be plasma amylase, which may be increased due to pancreatic damage resulting from the acidosis. Brown et al. (2000) identified plasma amylase as a criterion that could help to identify cattle that had experienced acute or subacute acidosis; elevated serum amylase appeared on the challenge day, and it remained higher for subacutely acidotic cattle than for unaffected cattle throughout the 14 d for which measurements were available. Serum amylase was also elevated in goats undergoing a severe acidosis challenge (Lal et al., 1991), but Krehbiel et al. (1995a) did not find any changes in plasma amylase in lambs that experienced a glucose-

induced ruminal acidosis. The severity of the acidosis challenge and the animal species and age may have contributed to the disparate responses in serum amylase.

Bacterial endotoxins have been identified as a mechanism through which acidosis harms the animal. Work as early as that of Dougherty et al. (1975a) demonstrated that endotoxin increased in the blood of cattle and sheep exposed to an acidotic challenge. More recent work demonstrated a relationship between ruminal endotoxin production in response to an acidotic challenge (increased grain in diet of forage-fed cattle) and subsequent response of cattle to the endotoxin as evidenced by increases in acute phase proteins (amyloid-A was more responsive than haptoglobin; Gozho et al., 2005).

Use of Acidosis Research Models

Any of the research models described above may be useful for studying various aspects of ruminal acidosis. Specific recommendations cannot be made with regard to the best way to develop a research model for studying acidosis. Instead, researchers need to consider what aspects of acidosis are most important to their hypothesis and develop a model accordingly.

In addition to factors such as feed intake, diet, and diet transitions, which were considered previously, acidosis models have been used to study a number of prevention strategies believed to affect acidosis. Studies have evaluated the effect of antibiotics on ruminal acidosis, with most designed to study agents that selectively reduce the growth of gram-positive, lactate-producing bacteria. The challenge models designed to develop an acute acidosis have been useful for assessing the effect of these antibiotics (Nagaraja et al., 1981, 1982, 1985; Coe et al., 1999), and step-up protocols (Galyean et al., 1992; Coe et al., 1999) and digestion studies (Zinn et al., 1994) have provided useful information. Similarly, acidosis models have been used to assess the impact of other feed additives, such as malate, that might stimulate lactate-utilizing bacteria (Montaño et al., 1999). Digestion studies with extensive ruminal measures have evaluated the impact of direct-fed microbial agents and yeast on ruminal acidosis (Ghorbani et al., 2002; Beauchemin et al., 2003). Hibbard et al. (1995) used several different acidosis challenge models to evaluate the effect that slaframine, a salivary stimulant, has on acidosis in cattle. Results of these various studies indicate that a number of antibiotics can limit the severity of ruminal acidosis, that malate and direct-fed microbial factors had little effect on ruminal acidosis, and that slaframine could lessen a challenge-induced decrease in ruminal pH when a subacute acidosis model was used, but not when an acute acidosis was induced.

Importance of Feed Intake

For an acidosis challenge model to be successful, enough carbohydrate needs to reach the rumen to induce acidosis, yet variable intakes by cattle do not always allow this to happen precisely. Direct placement of a carbohydrate challenge into the rumen ensures that intake is equalized, thereby reducing variation. However, as discussed above, the responses of cattle to feed that is consumed normally may be different than the response to a direct ruminal challenge. Responses of animals consuming feed would be expected to be similar to that observed under typical management conditions, thereby allowing development of a model that may closely mimic real-life situations.

It is difficult to develop severe acidosis in normally fed cattle, at least when intake is limited. This appears to be the case for most individually fed cattle used in research trials. In contrast, intake patterns of individual cattle maintained in a group pen can be more variable because the single animal has access to the feed offered to the entire pen. Eating behavior is also affected by effects of competition within group pen settings (Schwartzkopf-Genswein et al., 2003). Although intake patterns of individual animals housed in large group pens have not been well characterized, it is likely that a portion of the cattle will experience much larger variation in intake when housed together than when housed singly, such as in a typical research trial. This variation in the pen setting can be induced not only by access to a greater quantity of feed and effects of competition, but also by the possibility of feed deprivation during the previous day if other cattle consumed the offered feed first.

FUTURE DIRECTIONS FOR STUDYING RUMINAL ACIDOSIS

Although the study of the microbial alterations in ruminal acidosis began 5 decades ago, there are still major gaps in our knowledge of the microbial changes in response to changes in ruminal pH. Until the past few years, the methods for studying ruminal microbial flora, particularly for bacteria and fungi, have relied on traditional culture-based methods. However, there are inherent limitations imposed by culture-based methods that have precluded complete community analyses of the ecosystem. Although nucleic acid-based procedures, which are more sensitive and accurate, have been used to assess and monitor microbial changes in the rumen (Kocherginskaya et al., 2001; Zoetendal et al., 2004), there has been no published study of the microbial alterations in relation to ruminal acidosis. Such an approach would allow us to identify bacteria that may be numerically minor, but functionally have

a dominant role in causing acidosis. Tajima et al. (2000) monitored changes in the ruminal bacterial community structure in cows during transition from roughage to high grain by PCR amplification and sequencing of 16S rDNA clone libraries. Analyses of bacterial succession with PCR-derived libraries confirmed some known cultivation-based observations such as absence of ruminococci and prevalence of lactate-producing and utilizing bacteria during acidosis. However, the range of bacterial diversity observed was much higher than expected and a majority of the clones had unique sequences and no cultivated relatives. Therefore, research should be aimed at designing and applying molecular probes and techniques to facilitate isolation and quantification, to improve functional characterization, and to determine the contribution of ruminal bacterial species to ruminal acidosis.

Although there are a number of excellent models for studying many different aspects of ruminal acidosis in cattle, most of them suffer from an inability to mimic problems that exist in typical feedlot conditions. True incidence rates for acidosis are largely unknown and the effects of relatively mild cases of acidosis are not well understood. Subacute acidosis is defined as a situation in which no clinical symptoms are present, so it is not easily identified as being present or absent for any specific animal or group of animals. Both Galyean and Eng (1998) and Schwartzkopf-Genswein et al. (2003) suggested that feed intake patterns of cattle managed in a feedlot setting needed further study, as this would provide a baseline for understanding the variability in intake that can trigger acidosis. Both studies also suggested that relationships between feed intake patterns and metabolic disease (e.g., acidosis) need to be determined. Schwartzkopf-Genswein et al. (2003) particularly stressed the need to evaluate individual animals to identify unique characteristics that might predispose them to ruminal acidosis whereas others cope with ruminal acid production without obvious difficulty. We concur that feed intake patterns, ruminal pH, and health and performance of individual animals housed in feedlot conditions are key pieces of information needed to define both the incidence and importance of ruminal acidosis in the feedlot industry. Although currently expensive, measurement of feed intake, ruminal pH, and health and production of large numbers of individual cattle housed under feedlot conditions would provide great insight into the importance of ruminal acidosis. Technology is being developed to continuously monitor intraruminal parameters such as temperature and pH (Mayer et al., 2004; Penner et al., 2006), and feed intake by individual animals housed in group pens can be measured by technology such as the GrowSafe system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Additional technology will need to be used to analyze the large number of data points collected by these systems and subsequently determine what important relationships (if any) exist among the response variables. Because daily DMI, the pattern of feed intake, variation in the amount and pattern of feed intake, average ruminal pH, the pattern of ruminal pH over time, duration of suboptimal ruminal pH, and the variation in the pattern of ruminal pH may all be important criteria, it is likely that artificial intelligence (neural networks) or other advanced computational programs may be necessary to evaluate the importance of ruminal acidosis in affecting cattle performance as well as in defining the linkages between intake and acidosis.

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