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THE USE OF DH42, A PROPIONIBACTERIUM FOR THE PREVENTION OF LACTIC ACIDOSIS IN CATTLE

By

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ABSTRACT

THE USE OF DH42, A PROPIONIBACTERIUM FOR THE PREVENTION OF LACTIC ACIDOSIS IN CATTLE

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Propionibacteria have the ability to produce propionic acid from lactic acid. This characteristic was the basis for the hypothesis that if introduced into the rumen, lactic acid levels would be lowered thus, preventing ruminal acidosis. The effects of adding *Propionibacterium acidipropionici* DH42 to in-vitro ruminal fermentations at four different inoculation rates and to rumen fistulated beef cattle receiving a high grain finishing diet, via a rumen cannula and at inoculation rates higher than the in-vitro studies, were examined. *Propionibacterium acidipropionici* DH42 added to in-vitro fermentations resulted in no treatment effect ($P > .05$) on the levels of pH, lactic acid and volatile fatty acids produced. Animal studies revealed a significant treatment effect ($P < .05$) on rumen pH and blood pH. Blood and rumen lactic acid, total and individual volatile fatty acids, and the average dry matter intake of animals were not affected ($P > .05$) by the addition of strain DH42. Rumen and animal parameters in the treated and untreated groups resembled those pertaining to cattle experiencing subacute acidosis. Microbiological sampling of fermentors revealed growth of strain DH42 at 0 h only and absolutely no growth was detected during the animal trials. In conclusion, *P. acidipropionici* DH42 does not appear to have the ability to survive in the rumen and prevent the accumulations of lactic acid specifically when provided to cattle experiencing subacute ruminal acidosis.

To my Mother and my Father whom have shown an incredible amount of love throughout the years and always told me to put forth my best effort despite the obstacles that exist in life.

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Detours occur in life and we ask ourselves, "why?". Then, one realizes why. God knew what he was doing.....

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LACTIC ACIDOSIS

General description

Lactic acidosis in ruminants is a metabolic disorder often occurring in feedlot cattle, as well as, in other ruminants when a large influx of readily fermentable carbohydrates are suddenly consumed. It is characterized by a reduction in pH (4.0 to 5.0), increases in the concentrations of D (-) and L (+) lactic acids, and a disturbance in the microbial population of the rumen. The population changes from a predominantly gram (-) or lactate utilizing bacteria to a gram (+) or lactate producing organisms. In addition, ruminal protozoa concentrations are reduced as the acid load increases (Owens et al., 1998). The disorder can become systemic with changes in hematocrit levels (usually hemoconcentration), and decreases in the buffering capacity of blood due to the surge of lactate in the blood.

Energy intake and feed efficiencies are affected as decreased body weight and variable intake patterns are observed. Diseases such as rumen ulcers, parakeratosis, liver abscesses, and laminitis occur as a consequence of the acid accumulation in rumen. When severe, the disorder is an overt illness characterized by anorexia, depression, dehydration, recumbency, systemic acidosis, coma and death. Subclinical acidosis is less severe and more difficult to detect and may be considered subliminal at times. Animals experiencing subacute or subclinical acidosis often show no signs of an upset system. It is characterized as a less overt and insidious illness therefore, causing great economic losses to the industry in the forms of condemned livers, low weight gains, and wasted feed (Underwood, 1992; Nocek, 1997; Nagaraja and Chengappa, 1998).

Acidosis can occur in any breed of cattle. Nevertheless, Brahman cattle have been shown to be more susceptible to acidosis than the Angus breed and Holsteins appear to be the most resistant to the disease (Elam,1976). Weather changes, poor bunk management, accidental access to concentrate feeds (Elam,1976), and muddy pens or feedlots (Ritchie,1984) are some of the other critical factors that have been identified as related to acidosis.

Chronic and acute acidosis

There are two types of acidosis: chronic (subacute) and acute or clinical acidosis (Blezinger and Mies, 1990). The pH levels used to diagnose acidosis of the rumen are 5.6 and 5.2 for chronic and acute acidosis, respectively. (Cooper and Klopfenstein, 1996 [cited by Owens et al, 1998]).

Chronic acidosis does not produce visibly ill animals but sometimes does result in undetectable symptoms. It has been suggested (Britton and Stock, 1989) that variation in feed intake might possibly be the only indicator of subacute acidosis. In contrast, others have included a decrease in milk fat and milk yield (Allen and Beede, 1996) and a gray-green pasty to watery consistency of feces (Dirksen,1969) as other indicators of the disease. Animals suffering from chronic or subacute acidosis usually are alert and do not show a hesitation to move about (Dirksen,1969).

Acute or clinical acidosis is characterized as an illness following consumption of readily fermented carbohydrates in amounts sufficient to reduce pH of ingesta. The symptoms associated with this condition are total anorexia, increased water intake, incoordination, loose feces or diarrhea, grinding of teeth, restlessness, and apathy. Heart rate and rectal temperatures of cattle increase (100 to 140/min and 40 °C, respectively)

and respiratory rate increases to 40 to 60 breaths/minute (Dirksen,1969; Howard,1981). In addition, rumen motility also ceases (Slyter,1976; Elam,1976). As the disease progresses, body temperatures fall to hypothermic levels (98 °F to 100 °F) and hypoventilation, usually described as labored breathing, is evident with 10 to 20 breaths per minute (Howard, 1981). If not treated within 12 to 24 h after the onset of acute acidosis, recumbancy and coma can occur leading to eventual death (Johnson,1991).

Etiology of acidosis

A variety of feedstuffs are available that can cause lactic acidosis. The consumption of large amounts of grain, barley, corn , oats, and rye, or other sources of feeds that are high in sugar including sugar beets with the beet tops attached, potatoes, molasses, sugar cane, mangoes, and apples (Slyter,1976; Underwood,1992) induce acidosis. Other causative factors relating to occurrence of lactic acidosis is the industries ever present need to incorporate economics into feed management. The desire to optimize the rate of fermentation by making carbohydrates from feed more readily available allow for several feed processing techniques that besides having a positive effect on nutritional value, may actually have negative effects to ruminants. Those processing techniques include steam flaking, rolling, cracking, crushing or grinding and in relation to forages, their form and coarseness (Slyter, 1976; Dunlop,1972; Underwood, 1992). In a study relating to the steam flaking of sorghum grain and among others, its effect on subacute acidosis in feedlot steers, it was found that reducing flake density resulted in reduction of rumen pH and a propensity towards acidosis (Reinhardt et al., 1997).

Lactic acidosis is more evident in those animals switched from a high forage diet to a high concentrate diet. This was observed by Uhart and Carroll (1969), when they

changed ruminally fistulated steers on an all forage diet such as alfalfa, to a 90% grain diet. This abrupt change produced all steers to go off feed within 2 to 3 days. In contrast, though, incrementing the daily available concentrate causes animals to go off feed, as well as, the production of subtle symptoms. Lactic concentrations above 5 u*M* are indicative of acidosis and exceeding 40 m*M* indicate severe acidosis (Owens et al., 1998).

Complications

As a result of ruminal lactic acidosis, a sequel of other feedlot ailments occur that bring about a decrease animal performance. Theses include rumenitis, liver abscesses, laminitis, (Brent, 1976), grain bloat, and malabsorption (Britton and Stock, 1989).

The high acidity of lactic acid causes the rumen wall to become damaged. This allows for the systemic invasion of bacteria into the portal blood system of the animal causing deep ulcers in the rumen and abscessing of the liver thus, the terms rumenitis and condemned livers. The bacteria responsible for the invasion and colonization of the rumen wall is *Fusobacterium necrophorum* (Nocek, 1997; Nagaraja and Chengappa, 1998). *Actinomyces pyogenes* has also been implicated by others (Essig et al., 1988). This colonization eventually causes entry of these bacteria into the blood stream and ruminal wall abscesses. The bacteria present in the blood stream gain access to the liver by filtration causing infection and abscesses. It has been suggested that 12 to 32 % of beef cattle fed high or all concentrate diets may have abscessed livers (Brink et al., 1990).

Lactic acidosis has also been implicated in the incidence of laminitis in ruminants. Laminitis can occur in acute and subclinical or chronic acidosis (Allen and Beede, 1996; Nocek, 1997). Ruptured peripheral arterioles at the extremities caused by the release of histamine and endotoxins from dying cells due to a decrease in rumen pH from ruminal

lactic acidosis, may be responsible for this condition as some theories state (Brent, 1976; Mgassa et al., 1984). Signs of laminits seem to vary according to their severity. In severe conditions, feet are warm to the touch, animals will be reluctant to stand, and pain will be exhibited in all feet (Nocek, 1997). In chronic acidosis, animals will usually have overgrown hooves; characterized by becoming more elongated, flattened, and broadened (Nocek, 1997).

Another complication due to the onset of lactic acidosis includes a hardening of rumen epithelium also known as parakeratosis (Nocek et al., 1984; Dirksen, 1989). This usually occurs in mild, uncomplicated cases and causes a sloughing off of the epithelia on the rumen wall during low pH related to a lactic acidosis episode. The epithelia heals and forms blunt and clumped hardened papilla (Jensen and Mackey, 1979). This epithelial hardening has shown to reduce volatile fatty acid absorption (Huntington and Britton, 1979). In a recent study (Krehbiel et al., 1995), absorption of acetate was found to be 13% lower for lambs experiencing acidosis than the control group, leading the workers to conclude that acidosis affects the absorption of volatile fatty acids.

Bloat is caused secondary to the lactic acidosis syndrome in ruminants. Gas is normally produced in the rumen as a consequence of the fermentation process. Bloat is characterized by the accumulation of this gas within the rumen in amounts sufficient to increase pressure in the rumen. This pressure causes distention of the rumen. In addition, bacterial mucopolysaccharides such as slime can cause the ruminal contents to increase in their viscosity contributing to a frothy appearance (Cheng et al., 1976). This froth or foam prevents eructation adding to the distention of the rumen. Increased pressures in the abdomen and thoracic areas of the animal cause blood from the viscera to be moved into

the peripheral blood system depositing more CO_2 into the plasma thus, contributing to an already acidotic scenario (Blezinger and Mies, 1990).

The rumen microbial ecosystem and acidosis

The rumen is a highly complex and competitive microbial ecosystem. Population densities of 10^{10} to 10^{11} have been found for bacteria, 10^5 to 10^7 for protozoa and smaller amounts ($< 10^4$) of anaerobic fungi and facultatively anaerobic bacteria (Leedle, 1991; McAllister and Cheng, 1996). It has an internal temperature of 37 °C and pH under normal conditions of 6.8 to 7.0.

Rumen bacteria, in having the ability to ferment carbohydrates, are primarily responsible for causing lactic acidosis in ruminants. Under a well balanced hay to concentrate ratio, gram negative bacteria predominate (Dirksen, 1969). When the diet is abruptly changed to all concentrate, a large influx of carbohydrate is suddenly available to the rumen environment and gram positive bacteria dominate such as *Streptococcus bovis* and the lactobacilli (Dirksen, 1969; Slyter, 1976; Goad et al., 1998). *Streptococcus bovis* was first identified as playing an essential role in acidosis by Hungate in the 1950's and has been reviewed by others (Russell and Hino, 1985). Lactobacilli, have also been implicated as growing in combination with the *Streptococcus* (Dirksen, 1969). Krogh (1963), evaluated the rumen of cows and sheep suffering from acute acidosis during overfeeding and found a gram-positive flora predominating. The complete disappearance of protozoa and cellulolytic bacteria has been observed as pH decreases from 5.5 to 5.0 with an eventual cessation of the bacterial population at a pH of 4.0. In a recent study done by Goad et al. (1998), a comparison of two different types of diets revealed that amylolytic bacterial counts and lactobacilli counts were usually higher in grain adapted

steers after overfeeding with an all grain diet. In another study (Nagaraja et al., 1978), data suggested that feeding grain to an animal adapted to forage diet caused the onset of lactic acidosis, the proliferation of gram positive bacteria, the decrease in gram negative bacteria, as well as, an increase in endotoxin. Endotoxin production was thought to be a consequence of the lysis of gram negative bacteria and a subsequent decrease in their numbers (Huber, 1976). However, in vitro incubations showed that there was no decrease in the gram negative populations. Similar results were detected when after voluntary engorgement of goats with a 90% concentrate diet, decreases in gram-negative bacteria were not followed by increases in endotoxin concentrations (Suda, et al., 1997).

An overabundance of carbohydrate availability causes increases in the production of acids by rumen bacteria (Slyter, 1976). The production of these end products could be related to the growth of bacteria in the rumen (Dawson and Allison, 1988). When the growth rate of *Streptococcus bovis* is low, end products such as acetate and propionate are produced (Dawson and Allison, 1988). Nevertheless, when there is an abundance of a carbohydrate source and the growth is high, then lactic acid is produced. The growth rate, due to the excess availability of carbohydrates in the rumen environment, is not the only mechanism by which some bacteria multiply to produce lactic acid. Low pH in the environment has been found to trigger a shift in the metabolism of *Streptococcus bovis* towards producing lactate by changing its regulation of lactate dehydrogenase (Russell and Hino, 1985). This is known as the spiraling effect caused by *Streptococcus bovis.* This action is responsible for the lowering of the ruminal pH (Dirksen, 1969). Because they are sensitive to the acid they produce, they start to decrease in numbers and are eventually overcome by the growth of more acid tolerant lactobacilli. Lactobacilli are gram

positive rods which can ferment sugars to lactic acid and produce optimum growth at a pH of < 5.5 (Russel and Hino, 1985). Lactic acid produced by the lactobacilli is enough to cause a final pH of 4.5 causing decreases in other microbial populations in the rumen with an almost complete dominance of lactobacilli species in the rumen (Dawson and Allison, 1988). Nevertheless, the growth of the lactobacilli and the acid sensitivity of *Streptococcus bovis*, does not appear to be associated only with a decrease in *Streptococcus bovis* numbers. A recent study suggests the possibility of an antagonistic mechanism in the lactobacilli which could also be affecting the populations of *Streptococcus bovis* (Wells et al., 1997). In a study where there was a modest decline in pH (< 5.6), *Streptococcus bovis* diminished in numbers and the lactobacilli increased. It was suggested by the researchers that the pH alone could not account for the reduction in *Streptococcus bovis* and that certain strains of lactobacilli could possess certain bacteriocins, inhibiting their growth (Wells et al., 1997).

Gram negative bacteria including the lactate utilizers and protozoa decrease in numbers and eventually die (Slyter,1976) as the pH is affected by the heavy lactate production from *Streptococcus bovis* and the lactobacilli. Normally, a feeding of diets containing carbohydrates causes a temporary accumulation of lactic acid with eventual conversion to acetic and propionic acid by rumen microorganisms. However, lactate utilizing bacteria usually find difficulty in multiplying to effective numbers if there is a sudden surge of lactic acid to the system (Mackie and Gilchrist, 1978, 1979). *Megasphaera elsdenii* (considered by Slyter, 1976) as one of the most important lactate utilizers), produces propionate and is usually able to compete with the demand of lactate produced but only when the rumen is not overloaded with unreasonable amounts of

carbohydrates (Nocek, 1997). It utilizes 60 to 80 % of the lactate fermented in the rumen (Counotte et al., 1981) and makes up 20% of the lactate utilizers in ruminants fed high concentrate diets (Mackie et al., 1984). *Megasphera elsdenii* at 10^7 cfu/mL was able to increase pH and reduced lactic acid concentrations (> 3 m*M*) in ruminal fermentation studies in vitro involving other lactate utilizers (Hession and Kung, 1992). In another in vitro study done by Kung and Hession (1995), two levels of *Megasphera elsdenii* were tested on pH, lactate, and VFA concentrations. In relation to the lactic acid utilization, it was found that the two levels tested (10^6 and 10^5 cfu/mL) were effective in utilizing it. Other lactate utilizers found in the rumen include *Selemonas ruminantium*, *Veillionella alcalescens* and *Propionibacterium acnes* (Slyter, 1976).

Yeasts and anaerobic fungi make up part of the microbial population of the rumen. However, most of them are usually transient as they enter the rumen along with the feed. Several of these organisms have been found to ferment carbohydrates such as the anaerobic fungi *Neocalimastix frontalis*, and *Piromonas comunis*. The end-products produced by these fungi are lactate and acetate (Orpin and Munn, 1986 [as cited by Orpin and Joblin, 1988]). Yeast have also been found in the rumen and interestingly, they have been found surviving at the low pH produced during acidosis (Dirksen, 1969).

Lactic acid and VFA in ruminants

Under normal conditions, lactate does not accumulate in the rumen at levels exceeding 5 u*M*, but concentrations in the rumen of more that 40 m*M* indicate the onset of severe acidosis (Owens et al, 1998). Bacteria in the rumen produce two forms of lactic acid, the D(-) and the L(+) form. The L(+) form is identical to the lactate produce in muscle tissue from glucose and the D(-) form is not. At a high pH (6.0), 20% of the total

lactate produce is attributed to the D-isomer (Dawson and Allison, 1988). At a pH lower than 5.0 with lactate concentrations above 100 m*M*, the production of the D-isomer may be as high as 50% of the lactate produced (Dawson and Allison, 1988). Lactate in the rumen is produced by the catabolism of glucose via the Embden-Mayerhof-Parnas pathway as proposed by Mountfort and Robertson (1978) after extensive studies with pure cultures.

As mentioned previously, the enzyme responsible for the production of lactate in *Streptococcus bovis* is lactate dehydrogenase. When growth rates in bacteria are low, the concentrations of pyruvate within the cell are low and the enzyme is inactive. Pyruvate is then converted into acetate or propionate via the Embden-Mayerhoff-Parnas pathway. However, when energy is abundant and the growth rate of microorganisms intensifies, pyruvate increases and lactate dehydrogenase shifts production towards lactate. D(+) and L(-) lactate possess specific dehydrogenases. L(-) lactate is associated with L-lactate dehydrogenase which can be found in the cytosol of animal tissues and D-2-hydroxy-acid dehydrogenase which is associated with the D(+) lactate. Giesecke and Stangassinger (1980), found that D-lactate must pass the mitochondrial membrane before it could be oxidized by the D-2-hydroxy-acid dehydrogenase. The slow degradation of D-lactic acid and the toxicity it produces during lactate accumulation, could be attributed to its oxidation pathway (Dawson and Allison, 1988) and is responsible for the accumulation of lactic acid in the rumen of cattle experiencing acidosis (Slyter, 1976).

Volatile fatty acid production in the rumen is attained primarily by microbial fermentation. The primary volatile fatty acids produced in the rumen are acetic, propionic, and butyric acid (Gottschalk, 1986; Hungate, 1988). As the forage:concentrate ratio

decreases, the acetate:propionate ratio also decreases. For example, forage:concentrate ratios of 100:0, will give volatile fatty acid ratios of acetate, propionate, and butyrate of 71:16:7.9. When the forage to concentrate ratio is 50:50, the molar ratios of acetate, propionate and butyrate are 65.5:18.4:10.4. When there is more concentrate offered in the diet (80:20), the volatile fatty acid ratios would be 53.6:30.6:10.6 (Annison and Armstrong, 1970). In a study done by Goad et al. (1998) on hay adapted and grain adapted steers, ruminal acetate proportions decreased over time in both groups and was lower in the grain adapted steers at 60 h. The production of propionate increased with time in both groups and was higher in the grain adapted steers at 72 h post-feeding. Goad and Nagaraja (1988) have suggested that the volatile fatty acids may be responsible for the low pH seen in subacute acidosis because lactate levels were low (< 5 m*M*) in contrast to acute acidosis where lactate is seen in greater concentrations. Krehbiel et al. (1995), studied the effects of increasing severity of acidosis on rates of absorption of ruminal volatile fatty acids and found that the concentrations of ruminal volatile fatty acids in lambs intraruminally dosed with glucose responded individually as the amounts of glucose (0, 6, 12, and 18 g/kg) increased. Acetate concentrations decreased (63.3, 46.6, 37.5 and 37.7 m*M*), as glucose dose increased, propionate showed an increase of 21.0 and 43.6 m*M* at 0 and 6 g/kg, respectively with a decrease from 12 to 18 g/kg of glucose infused. Butyrate tended to decrease (15.0, 8.5, 7.5 m*M*) when glucose was infused at 0, 6, and 12 g/kg and increased (14.0 m*M*) at 18 g/kg infused glucose. Amongst the parameters studied by Patra and Swarup (1996) on studies with experimental acidosis in sheep, total volatile fatty acid concentrations abruptly increased from 44.16 mmol $\cdot L^{-1}$ to 83.00 mmol $\cdot L^{-1}$ in 12 h after overfeeding. They attributed this to the rapid fermentation by the

starch degrading bacteria of the rumen (as discussed previously). Total volatile fatty acids 24 h after the engorgement, decreased to 57.60 mmol $\cdot L^{-1}$, leading them to deduce that the decrease in total volatile fatty acids was caused by an increased absorption rate at a low ruminal pH.

Before volatile fatty acids can be produced, carbohydrates must first pass through the pyruvate intermediate. End product formation primarily depends on the type of structural carbohydrate fermented and the type(s) of bacterial species involved in the fermentation process.

Acetate production is formed by the pyruvate-formate lyase system. In this system, formate and acetyl-coenzyme A (CoA) are formed as the intermediates. The formate is converted to carbon dioxide and H_2 by other bacteria. This is the main pathway for acetate production in the rumen (Fahey and Berger, 1988). Another pathway involved in the production of acetate in the rumen utilizes pyruvate ferredoxin oxidoreductase. It produces ferredoxin, which is the acceptor for some bacteria (Glass et al., 1977), carbon dioxide and acetyl-CoA. In either pathway, the resulting end product is acetate and bacteria such as *Megasphera elsdenii* and *Veillonella alcalescens* have been observed utilizing the ferredoxin-linked pyruvate oxidoreduction pathway (Fahey and Berger, 1988; Russell and Wallace, 1988).

Propionate production is primarily via the dicarboxylic acid pathway. The decarboxylation of succinate by *Selenomonas ruminantium* is the primary method of propionate production in the rumen of cattle (Wolin and Miller, 1988). It must first produce succinate as an intermediate. A carboxyl group is then removed from the succinate by succinyl-coenzyme A ultimately leading to the production of propionate.

Nevertheless, there is an alternate pathway for the production of propionate. It has been named the acrylate pathway and bacteria such as *Megasphera elsdenii* have been implicated in utilizing it for the production of propionate (Paynter and Elsden, 1970). In this pathway, succinate nor succinyl-CoA are used as intermediates. Instead, pyruvate is converted to either form of lactate (L-, D-, or DL-) which is then converted to acrylyl-CoA and reduced to propionyl-CoA (Gottschalk, 1986). One third of the total propionate produced is via the acrylate pathway (Fahey and Berger, 1988). Nonetheless, the production of propionate is of vital importance to the ruminant because of its involvement in gluconeogenesis. Propionate is the only volatile fatty acid which makes contribution to production of glucose in the ruminant animal (Fahey and Berger, 1988; Wolin and Miller, 1988). One study suggested that 27-54% of glucose is produced from propionate (Lindsay, 1970).

Butyrate production is mainly via the reversal of the B-oxidation pathway (Fahey and Berger, 1988). In this pathway, acetacetyl-CoA, L(+)-B-hydroxybutyrly-CoA, and crotonyl-CoA are intermediates with butyryl-CoA being formed proceeded by butyryl phosphate; thus giving rise to butyrate (Gottschalk, 1986).

METHODS UTILIZED IN MANIPULATING RUMEN FERMENTATION

Introduction

The growing public concern over the use of antibiotics in the animal feed industry to control rumen fermentation, diseases, and improve animal efficiency has lead researchers to probe into the use of less artificial methods in animal feeds. Antibiotics have been used as feed additives for farm animals since the second world war (Pusztai, et al., 1990). It has been the repeated evidence of their beneficial effects on the growth and

health of livestock that has made them a popular choice in the industry. Nevertheless, the use of antibiotics is still widely accepted in the industry but carries the risk of bacterial strains becoming resistant and therefore affecting future therapeutic uses in both man and animal. As pressure by the public, media and the medical community increases, it would be correct to state that in the 21st century, the world could be seeing the elimination of the use of antibiotics in all livestock feed with an increase in the use of live feed supplements such as rumen bacteria, fungi and yeast.

In the next section, the various methods or additives used to modify rumen fermentation activities and its microbial population along with the use of antibiotics will be discussed.

Ionophores and non-ionophores

There are two types of antibiotics which are used in the livestock feed industry: ionophore and non-ionophore antibiotics. Ionophore antibiotics are bacteriostatic and not bactericidal (Nagaraja and Taylor, 1987). They are known for their ion bearing properties (Bergen and Bates, 1984 [as cited by Wallace, 1992]), and it is their ability to permeate the cell envelope of different species to different extents that is the basis of their selective action (Wallace, 1992) and bacteriostatic activity. An example of an ionophore would be monensin as a Na^+ , K^+/ H^+ antiporter (Wallace, 1992). Non-ionophore antibiotics do not act on cation transport and differ in their chemistry (ie. glycopeptide, tetracycline, polypeptide), antibacterial spectrum, molecular weight and if they are absorbed from the gut or not (Nagaraja, 1995). For example, avoparcin is a glycopeptide which inhibits cell wall synthesis by inhibiting the incorporation of *N*-acetylglucosamine into the peptidoglycans of the bacterial cell walls (Wallace, 1992; Jouany, 1994).

The effectiveness of using ionophores is in an increased efficiency of feed conversion and an improvement in the rate of gain (Bauer et al., 1992; Herold et al., 1994; DiConstanzo et al., 1997). These two effects are primarily attributed to the changes in ruminal fermentation patterns as a consequence of the incorporation of ionophores (Nagaraja, 1995) in feeds. According to Bergen and Bates (1984), amongst the three major areas associated with ionophore feeding are: increased production of propionate and decreased lactic acid production and froth formation in the rumen leading to decreased ruminal upsets. The increased production of propionate by the use of ionophore feeding may offer the animal considerable advantage over other end products such as acetate because of its involvement in gluconeogenesis. There have been various studies where the use of ionophores has lead to an increase in propionate production. Nagaraja et al. (1981) induced acidosis in sheep treated with either lasalocid or monensin and found an increase in propionate in treated animals after grain engorgement. In-vitro studies with salinomycin was shown to enhance propionate production, but in-vivo studies in cattle using the same ionophore, showed that propionate increased for 48 h then gradually declined (Nagaraja et al., 1985). Monensin in cattle has also shown to be beneficial in propionate production (Burrin and Britton, 1986) and in-vitro fermentation studies of soluble carbohydrates treated with monensin caused a 40% increase in molar proportions of propionate (Tung and Kung, 1993). In contrast, studies with laidlomycin propionate reported no significant influence on concentrations of volatile fatty acids including propionate (Herold, et al., 1994; Bauer et al., 1995). It is thought that the increase in propionic acid is due to a redirection in the utilization of hydrogen and a decrease in the production of methane (Nagaraja, 1995; Jouany, 1994) in animals fed ionophores.

Ionophores have also been shown to reduce ruminal upsets. Because cereal grain is one of the major components in feedlot diet, it has had a notoriety for causing situations in which rapid fermentation of the feedstuffs has lead to the accumulation of organic acids in the rumen. Lactic acidosis causes major economic loss to the ruminant livestock industry. Ionophores are selective toward gram positive microbes and the major lactate producing bacteria such as *Streptococcu bovis* and the *Lactobacilli* spp., but do not affect the lactate utilizers (Dennis et al., 1981). Nagaraja et al. (1985) have reported significant reductions in lactate production and acidosis when ionophores have been administered during a high carbohydrate diets. In a study done by Nagaraja et al. (1981), lasalocid and monensin were administered into the diet and their effects on lactic acidosis in cattle were observed. Antibiotic treated cattle showed higher rumen pH and lower L (+) and D (-) lactate concentrations. Interestingly, administering the antibiotics 7 days before experimentally inducing acidosis with corn (27.5 g/kg body weight) prevented acidosis while administering antibiotics 2 days before the glucose induced acidosis was not enough to prevent acidosis. The authors suggest this might be due to the differences in carbohydrates used. Salinomycin was compared against the popular monensin and lasalocid in preventing experimentally induced lactic acidosis (Nagaraja et al., 1985). All showed an increase in pH and a decrease in the lactate isomers, but salinomycin was shown to be a least 3 times more powerful than monensin or lasalocid. Monensin alone was studied to evaluate its response in cattle to subacute acidosis (Burrin and Britton, 1986). In this study, monensin was able to maintain a higher ruminal pH by reducing concentrations of volatile fatty acids. Subacute acidosis is characterized as a less severe

acid load with increases in volatile fatty acids and is thought to be the most common form of acidosis in grain fed ruminants (Nagaraja, 1995).

Ionophore antibiotics are extremely effective against gram positive organisms and impose no effect on gram negative bacteria. This was the outcome of an in-vitro study conducted by Cheng and Wolin (1971). In this study the effect of monensin and lasalocid-sodium on the growth of gram positive and gram negative bacteria was evaluated. The results showed that *Bacteroides ruminicola* and *Selenomonas ruminantium*, two gram negative rumen bacteria, were insensitive to either ionophore causing their proliferation. The gram positive organisms utilized in this study, *Ruminococcus albus,* and *R. flavefasciens,* were inhibited and *Butyrivibrio fribisolvens,* although possessing a gram negative stain, but having gram positive cell wall structure, was inhibited as well. It was also suggested by these workers that the selection of monensin and lasalocid-sodium for gram negatives that produce succinate to propionate could lead to a beneficial propionate formation in the rumen. Equally postulated, selection against the hydrogen and formate producing gram positives used in this study, would lead to a decrease in the undesired and wasteful methane production in the rumen.

In essence, ionophore antibiotics inhibit gram-positive bacteria such as *Lactobacillus* and *Streptococcus* and those that have a gram-positive cell wall structure but stain gram-negative such as *Butyrivibrio* and *Ruminococcus*. Gram-negative bacteria such as *Megasphaera, Ruminobacter, Selenomonas, and Veillonella* species, are usually resistant to ionophores. The ruminal bacteria sensitive to ionophores produce lactate, butyrate, formate or hydrogen and the bacteria which are resistant produce succinate or

propionate as the end products of fermentation (Cheng and Wolin, 1979; Nagaraja and Taylor, 1989 [table 5; as cited by Nagaraja, 1995]).

Non-ionophore antibiotics are antibiotics which do not act on cation transport across the cell membrane, as mentioned previously, yet their antibiotic activity is on the inhibition of cell wall synthesis (Wallace, 1992). Such antibiotics include avoparcin, virginiamycin, thiopeptin, and tylosin. Nevertheless, in order for these antibiotics to be accepted in livestock feed, they must not be absorbed from the gut or partially absorbed at a low dosage; primarily because of the residues in milk and meat and because a withdrawal time would not be required. The molecular weight of these non-ionophore antibiotics have much to do with their absorption from the gut. For example, avoparcin, because of its molecular weight in excess of 1000 (Table 13.8; Nagaraja, 1995), would not be absorbed from the gut and thus, would not require a withdrawl period. In contrast, virginiamycin, because of a molecular weight of 525 (Table 13.8; Nagaraja, 1995), would require a withdrawal period because of the antibiotic residues that would be detected in either milk or meat products.

Non-ionophore antibiotics generally improve growth and enhance feed efficiency and as in ionophore antibiotics, the response is mainly attributed to its effect on the rumen flora. There have been many modes of action postulated (Nagaraja, 1995) to account for the growth effect that these antibiotics have on ruminants. One mode of action pertains to the modification that non-ionophore antibiotics have on rumen fermentation. These act directly on the microbial population to improve the fermentation efficiency. This is an important factor when relating to digestive upsets in the rumen such as lactic acidosis from the feeding of starch rich feeds. Muir and Barreto (1979) tested several antibiotics in their

ability to inhibit *Streptococcus bovis*. Amongst the many tested, thiopeptin was among the most effective antibiotics at inhibiting this microorganism. Thiopeptin is a sulfur-containing peptide produced by *Streptocmyces tateyamensis* and has been shown to possess strong activity against gram positive bacteria (Miyari et al., 1972). This prompted Muir et al. (1980) to study the induced ruminal lactic acidosis in sheep fed wheat with or without thiopeptin and related antibiotics. Their results indicated that thiopeptin given in a single dose along with the wheat on the day of the challenge, prevented lactic acidosis completely as it reduced rumen lactate concentrations by 80 to 90%. They also noticed a normal rumen fermentation pattern during wheat supplementation with thiopeptin as an increase in volatile fatty acids were noted. Penicillin was also tested for its efficiency in preventing acidosis but was not found to be effective because of its broad spectrum antimicrobial activity affecting even some gram negatives (Flulghum et al., 1968 [as cited by Muir and Baretto, 1979]).

Virginiamycin is a fermentation product of *Streptomyces virginiae*. It has been shown to have antibiotic effects against gram positive bacteria (Nagaraja et al., 1987). A single drench of virginiamycin was evaluated in two trials for its ability to protect sheep against lactic acidosis when fed wheat *ad libitum* (Thorniley et al., 1998). In this study, an increase in pH was observed in sheep drenched with virginiamycin than in the control sheep with an increase in weight and propionate concentrations were increased on the day after drenching and exposure to the wheat while acetate and butyrate decreased. The workers in this study concluded that sheep drenched with virginiamycin at 40 to 80 mg did not become acidotic and that a single drench of the antibiotic was able to effectively prevent lactic acidosis. Ruminal bacterial species were tested for their susceptibility and

resistance towards virginiamycin in studies done by Nagaraja (1987). In this study, virginiamycin was inhibitory to the two major lactic acid producing bacteria, *S. bovis* and *Lactobacillus* sp., responsible for lactic acidosis in ruminants (Slyter, 1976). The ability for virginiamycin to inhibit lactic acid production in vitro was again confirmed in a study done by Nagaraja et al. (1987). In this study, viginiamycin inhibited lactic acid production by 90 to 93% at the highest concentration (24.0 *u*g/mL), but decreased propionate production when concentrations were < 6.0 *u*g/mL .

Avoparcin, produce by the yeast *Streptomyces candidus*, inhibits gram-positive bacteria as it disrupts the peptidoglycan synthesis of the cell wall. It is presently not approved in the United States, but may be used in Europe (Nagaraja, 1997). Nevertheless, in a study done by Nagaraja and Taylor (1987) avoparcin, was found to be less inhibitory to *S. bovis* than the other non-ionophore compounds except tylosin. They suggested that avoparcin might not be active against all gram-positive bacteria (Walton, 1978 [as cited by Nagaraja and Taylor, 1987]). Nagaraja et. al (1987) reported that out of the non-ionophores tested, avoparcin increased the molar proportion of propionate with increasing concentration (*u*g/mL) and was the least effective in reducing lactic acid concentrations. This is in contrast to the study done by Nagaraja and Taylor (1987) which reported a less inhibitory effect of avoparcin towards lactic acid producing bacteria. Because avoparcin is also favored as a growth promotant, Dyer et al. (1980), evaluated amongst the many parameters, growth rate and volatile fatty acid concentrations in cattle fed a 77% barley diet. They reported cattle consuming less feed per unit gain ($P < .05$) than control cattle and reported a trend in the increase of propionate with increasing levels of avoparcin.

Tylosin is produced by *Streptomyces fradiae* (Berkman et al, 1961 [as cited by Nagaraja, 1997]). Its antibiotics effects are on gram-positive and some gram-negative bacteria (Nagaraja and Taylor, 1987). Tylosin in combination with monensin is used in the cattle industry for reducing the incidence of liver abscesses. The organism attributed to the onset of liver abscesses is *Fusobacterium necrophorum*. The organism is gram-negative and tylosin has the ability to destroy some gram negative organisms as mentioned. Tylosin showed some activity towards gram positive lactate producers such as *S. bovis* and *Lactobacillus* sp. although not as strong as some other antibiotics tested (Nagaraja and Taylor, 1987; Muir and Baretto,1979). Tylosin showed an extreme effectiveness in reducing lactic acid accumulation in vitro and showed a tendency to increase the molar proportion of propionate. In contrast, tylosin at high concentrations (>6.0 *ug*/mL) decreased the proportion of propionate. In studies with feedlot steers fed a 60% barley diet, Norton and Nicholson (1980) reported a decrease ($P < .05$) in liver abscesses in steers fed tylosin, no beneficial effects of the antibiotic on propionate concentrations, and no major effect on gain or feed efficiency. This lead the researchers to conclude no beneficial effects in using tylosin in the diets under their experimental conditions. In contrast, the feeding of tylosin has been shown to increase weight gain and feed efficiency in feedlot cattle (Potter et al., 1985).

Microbial feed additives

Much confusion has been associated with the use of the popular term "Probiotics" in reference to bacteria, yeast and fungi (Martin and Nisbet, 1991). Nevertheless, in light of the confusion, the Food and Drug Administration in 1989, required manufactures to use the term Direct Fed Microbials (DFM) and defined DMF's as "a source of live (viable)

naturally occuring microorganisms"; this includes yeast, fungi, and bacteria (Miles and Bootwalla, 1991 [as cited by Martin and Nisbet, 1991]).

Much work has been done with yeast and fungal cultures in benefiting the microbial fermentation and efficiency in the rumen and has been reviewed extensively (Williams and Newbold, 1990; Martin and Nisbet, 1991; Wallace, 1992; Kmet et al., 1993; Jouany,1994; Newbold, 1995; Yoon and Stern, 1995; Girard, 1996; Chesson and Wallace, 1996). Yeast and fungi supplementation is primarily utilized in adult ruminants because of their effects on feed intake and in preventing lactic acidosis (Chesson and Wallace, 1996). However, the two main organism commonly used have been *Saccharomyces cerevisiae* and *Aspergillus oryzae* (yeast and filamentous fungus, respectively) (Chiquette, 1995).

Harrison et al. (1988) utilized a yeast culture supplement composed of *Saccharomyces cerevisiae* to evaluate its effect on ruminal metabolism and digestibility in dairy cattle. Amongst the parameters observed in this study, a low pH was reported along with increases in the molar proportions of propionate. Anaerobic bacteria and cellulolytic bacterial counts also showed an increase in the supplemented cows as opposed to the controls. However, an in vitro study by Kung et al. (1997) revealed that supplementing continuous fermetors with *Saccharomyces cerevisiae* on a 50:50 concentrate:forage diet, showed no effects on major fermentation acids (acetate and propionate) nor on the pH.

The in vitro effects of this yeast's ability to influence lactate utilization of ruminal bacteria have been studied. *Saccharomyces cerevisiae* (Levucell SC) was evaluated for its effect on lactate metabolism by *S. bovis* and *M.elsdeni* (Chaucheyras et al., 1996). In this study, the growth of the yeast and *S. bovis* in coculture reduced the lactate production by the bacteria, suggesting less glucose availability for the bacteria. *Saccharomyces*

cerevisiae stimulated the utilization of L-lactate by *M. elsdenii* with the effect dependent on the concentration of yeast used. When *S. bovis* and *M. elsdenii* were grown in the presence of *S. cerevisiae*, lactate reduction was improved. The fermentation products produced by the bacteria were also increased in this study as a consequence of the yeast. Researchers concluded *S. cerevisiae* to be effective in reducing lactic acid accumulation in vitro.

It has been suggested that the mode of action of yeast in stimulating ruminal fermentation is due to the removal of O_2 from the ruminal environment which would stimulate the growth of the strictly anaerobic bacteria (Rose, 1987 [as cited by Newbold et al, 1996]). Newbold et al. (1996) investigated this theory and reported that *S. cerevisiae* and a commercial preparation Yea-Sacc, increased the rate of oxygen disappearance from fermentors by between 46 and 89%.

The combination of *S. cerevisiae* and *A. oryzae* have been studied by some to varying extents (Chiquette, 1995). When *S. cerevisiae* and *A. oryzae* were used alone or in combination as a feed supplement for beef and dairy cattle, higher concentrations of acetate, propionate and total volatile fatty acids were reported and a lower pH was reported. In this study, bacterial counts were not affected nor their ability to colonize feed particles. The addition of *S. cerevisiae* did not affect milk yield but the combination of the yeast and fungi showed a positive effect.

Bacterial probiotics are primarily used in young ruminants principally during their early days of life in order to reduce or prevent digestive upsets and promote the development of normal rumen anaerobic flora (Kmet et al., 1993). The primary bacteria used in feed for calves are microorganisms such as *Lactobacillus*, *Enterococcus*,

Bifidobacterium, and *Bacillus* spp. For example, in a study done by Bondai et al. (1986), the incidence of diarrhea was reduced and a decreased count of fecal coliforms in the intestines were reported in studies by Gilliand et al.(1980) when *Lactobacillus acidophilus* was used in calves. Beeman (1985), tested the effects of *Lactobacillus acidophilus* on Holstein male calves with a history of diarrhea, and reported weight gains of 8 kg as opposed to the 3.5 kg gain by control calves two weeks after the treatment with the lactobacilli. Lactic acid bacteria have been suggested to have pH lowering effects and alterations in the fermentation activities in the rumen of young ruminants (Kmet et al., 1993; Chesson and Wallace,1996). For example, young Holstein calves benefited from a yogurt containing *L. acidophilus* supplement as they showed rumination activities at 30 days after birth (Chesson and Wallace, 1996). Pollman (1985) evaluated the use of a probiotic product containing *L. acidophilus* on the weight gain of calves convalescing from neonatal diarrhea when treated with antibiotics. It was noted that on average, weight was increased 17.7 lbs (8 kg) in treated animals as compared to 7.8 lbs (3.5 kg) in control calves and coats were healthier in appearance. Lactic acid is used by the calf as a nutrient and it promotes a decrease in the pH of the intestine making it a less desirable place for pathogenic organisms such as *E. coli* to grow (Pollman, 1985).

Although mainly fungal additives are primarily used in adult ruminants (Kmet et al., 1993; Yoon and Stern, 1995), recent attention has turned to the use of bacterial organisms to enhance ruminal microbial fermentations and performance. Wiryawan and Brooker (1995) were able to control acute lactic acidosis in sheep when an inoculum of 10^8 cfu of *Selenomonas ruminantium* preceded an acute grain feeding and the effect of combining *Selenomonas ruminantium* and *Megasphaera elsdenii* was shown to be even

more beneficial in combating the effects of an acidosis challenge. In this study, pH remained high and rumen lactate levels were undetectable in sheep treated with *Selenomonas ruminantium*. In sheep treated with both microorganisms, marked increases in acetate, propionate and butyrate were observed. Huffman et. al. (1992) evaluated the use of *Lactobacillus acidophilus* in steers experiencing subacute acidosis. A 10^8 cfu/d inoculum reduced the amount of time that ruminal pH was below 6.0. In contrast, Klopfenstein et al (1995), reported that *Lactobacillus acidophilus* had no influence on subacute acidosis in cattle. The effect of *Megasphaera elsdenii* in preventing lactate accumulations in vitro was evaluated in a study done by Kung and Hession (1995). A high and a low dose of *M. elsdenii* revealed its ability to prevent an accumulation in lactic acid and excessive drops in pH. The low dose consisting of 8.7×10^5 cfu, showed lactate concentrations of 5 m*M* by 7 hours of fermentation and the high dose (8.7×10^6 cfu) held steady at 2 m*M*. Propionate and acetate concentrations decreased after 4 h of fermentation and butyrate increased. Additionally, the ability of *M. elsdenii* to withstand an additional substrate challenge was evaluated as half of the fermentation media was inoculated into a fresh substrate without the addition of *M. elsdenii*. Lactate concentrations remained low and it was concluded that *M. elsdenii* has the potential to prevent the accumulation of lactic acid in high carbohydrate diets. In a study done by Hession and Kung (1992), *M. elsdenii* and *Propionibacteria shermanii* were evaluated for their effects on rumen fermentation. In their preliminary studies, it was observed that high (10^9 cfu/mL culture) and low (10^{10} cfu/mL culture) inocualtion rates of *P. shermanii* tended to decrease L-lactic acid concentrations when compare to untreated cultures, but pH remained low. When *P. shermanii* was inoculated at 10^8 cfu/mL culture pH remained

at 4.82, lactic acid levels rose to 30 m*M*, and higher levels of propionic and acetic acids were reported. Inoculations of *M. elsdenii* at 10^6 cfu/mL culture showed and increase in pH (5.4) five hours after treatment with lactic acid levels at 25 m*M* and after 7 h decreased to less than 5 m*M*. *M. elsdenii* at 10^7 cfu/mL culture held pH at 5.43 after 5 h of treatment and lactic acid levels remained at 3 m*M* throughout the entire experiment. It was concluded that *P. shermanii* would not be acceptable for treating acidosis because it has a tendency to decrease pH due to the production of propionic and acetic acids. *M. elsdenii* would be a much better choice because of its production of weak acids that would moderate the decrease in pH. *M. elsdenii* was also tested in vivo in a trial conducted by Robinson et al. (1992). In this experiment, *M. elsdenii* was used to test its effectiveness in preventing acute acidosis and in enhancing feed intake in cattle. *M. elsdenii* was inoculated in rates of 10^{12} cfu/animal for three consecutive days before during and after the acidosis producing feed was fed. It was found that feed intake was increased 24% more than untreated animals, and workers found significant interactions between treatments and days ($P < 0.2$) for ruminal pH, lactate, butyrate, valerate and total volatile fatty acids. It was concluded that using *M. elsdenii* as an inoculant can accelerate the adaptation of ruminants to high concentrate feeds.

Recently, attention has been brought to the possible manipulation of the rumen microbial ecosystem with bacteriocins as another means of improving ruminant production (Teather and Forster, 1998). Bacteriocins are proteinaceous "antibiotics" produced by some bacteria (Teather and Forster, 1998) which have a common mode of action; to interfere with energy transduction and membrane transport processes (Montville and Bruno, 1994). Most of what is known about them comes from gram (+) bacteria and they

are very resistant to inactivation unlike proteins; they are active at pHs as low as 2 and at temperatures as high as 100 °C (Jack et al., 1995).

There has been limited work done in discovering rumen organisms capable of producing bacteriocins. Iverson and Mills (1976) suggested that *S. bovis* could be a probable producer of a bacteriocin. *Staphylococcus* and *Enterococcus* isolated from calves were also shown to posses bacteriocin properties (Laukova et al., 1993; Laukova and Marekova, 1993). *Ruminococcus albus* has also shown to produce a bacteriocin like inhibitor (Odenyo et al., 1994). Nevertheless, these results suggest the presence of bacteriocins in the rumen. After an extensive survey of 50 *Butyrivibrio fibrisolvens* isolated, 25 were found to have some sort of bacteriocin activity and one strain, AR10, has been completely defined as having its bacteriocin like activity in a single peptide with a molecular weight of approximately 4000 Da (Kalmokoff and Teather, 1996). It has been named Butyrivibriocin AR10 and represents the first bacteriocin isolated from a rumen anaerobe (Teather and Forster, 1998).

THE PROPIONIBACTERIA

General characteristics

Propionibacteria belong to the recently proposed class *Actinobacteria* (Stackenbrandt et al., 1997). They are generally described as pleomorphic rods with one end rounded and the other tapered or pointed. The cells tend to arrange themselves in V or Y configurations and are characteristically described as "Chinese characters". They may be arranged in pairs, short chains, or may occur singly.

Propionibacteria stain gram positive. They are non-motile, non-sporeforming, catalase-positive, facultative anaerobes. Their catalase-positive reaction is contradictory

to what is generally observed in anaerobic bacteria; anaerobic bacteria are usually catalase-negative, but the former has been shown to occur (Vorob'eva et al., 1968 [as cited by Hettinga and Reinbold, 1972]). Cell colonies may appear white, gray, red, yellow, cream or orange and the temperature range at which cells have been observed to grow rapidly is between 30 to 37 °C (Cummins and Johnson, 1986).

Propionibacteria are divided into two groups according to their habitats: the classical and the cutaneous strains. The classical strains include those bacteria found in cheese and dairy products (Grappin et al., 1999). However, they have also been found in silage fermentations and in fermenting vegetables (Babuchowski, et al., 1999; Merry and Davies, 1999) and are known as *Propionibacterium freudenreichii, P. jensenii, P. thoenii,* and *P. acidipropionici.* The cutaneous strains include those bacteria that are found on human skin. One acne bacillus, originally described as a *Corynebacterium* is an example of this group and is referred to as the "acne group strains" or the "cutaneous propionibacteria (Cummins and Johnson, 1986). These cutaneous bacteria are known as *Propionibacterium acnes, P. avidum,* and *P. granulosum.*

There are distinctive characteristics amongst the morphology of these two types of propionibacteria. The classical propionibacteria tend to exhibit a shorter and thicker rod while *P. acnes* tends to show a longer and slender irregular rod (Cummins and Johnson, 1986). Nevertheless, there may be variability in the morphology from strain to strain especially in the early log phase. For example, strains of *P. acnes* may be long and irregularly slender (as discussed previously) in a young culture (early log phase), but in older (post log phase) cultures, strains are more coccal (Cummins and Johnson, 1986).

Metabolic pathways

The metabolism and growth of propionic acid bacteria has been reviewed extensively by Hettinga and Reinbold (1972 a, b). Fermentation products of propionibacteria include large amounts of propionic acid, acetic acids, and lesser amounts of formic, succinic, lactic acid and carbon dioxide. The earliest study regarding the products of the fermentation of propionic acid was conducted by Fitz in the 19th century (Wood, 1981). He proposed that 3 moles of lactate would yield 2 moles of propionate, 1 mole of acetate, 1 carbon dioxide, and 1 water. This stoichiometry has withstood the test of time as Gottschalk (1986), proposed that many anaerobic bacteria ferment glucose to propionate, acetate, and carbon dioxide and that a preferred substrate of propionate-forming bacteria is lactate; the fermentation products being as described by the Fitz equation discussed above.

Piveteau (1999), in reviewing the metabolism of lactate and sugars by dairy propionibacteria, discussed the production of propionate and the transcarboxylase cycles. He suggested that the production of propionate involves reactions arranged in several cycles. First, a transcarboxylation occurs in which the COOH group of methylmalonyl-CoA reacts with pyruvate thus forming oxaloacetate and propionyl-CoA. The oxaloacetate is then reduced to succinate via malate and fumarate. Succinate is then converted to propionate via methylmalonly-CoA intermediates such as succinyl-CoA and propionyl-CoA. The cycle is completed when a carboxyl group is removed from methylmalonyl-CoA and transferred to pyruvate to yield oxaloacetate. A second transcarboxylation reaction can also be produced during propionate production, as methylmalonyl-CoA is regenerated from succinyl-CoA and reacts with a new molecule of

pyruvate. According to Piveteau (1999), the recycling of CoA and the minimal carbon dioxide fixation produced by the transcarboxylation reaction, are beneficial to the production of propionate as there is a minimum loss of energy involved. This pathway has also been called the succinate-propionate pathway as described by Gottschalk (1986). This is the most widely used pathway by the propionibacteria.

There is another process unique to propionibacteria which involves the anaerobic fermentation of erythritol. It was reviewed by Hettinga and Reinbold (1972) and briefly mentioned by Wood (1981). Wood and Leaver (1953) proposed that the main products from the fermentation of erythritol by *Propionibacterium pentosaceum* were propionic, acetic, formic and succinic acids.

Substrate preference, propionic acid production, pH, and temperature requirements of propionibacteria

Piveteau (1999) discussed the utilization of various substrates during propionic acid fermentation. He also mentioned that more ATP was produced from sugars than from lactate and that as a consequence, increased growth rates and cell yields were obtained during the fermentation of lactose, glucose, and galactose than from lactate. Nevertheless, when lactate is fermented, more propionate and acetate, are produce per mole of pyruvate than when any of the above mentioned sugars are used (Babuchowski et al., 1993).

Piveteau et al. (1995) reported a preference towards lactate utilization in culture when propionibacteria were incubated in a whey medium containing lactose and Marcoux et al. (1992) observed lactose utilization in a whey-based media, but only when the majority of the lactate had been utilized. The growth *P. acidipropionici* on a mixture of

glucose and lactate was determined in studies by Perez-Chaia et al. (1994). It was found that glucose was utilized first followed by a shift in the substrate utilization to lactate when glucose levels were low. In contrast, Lee et al. (1974) reported the opposite occurring as lactate was utilized first by strains of *P. shermanii* . In a study done by Babuchowski et al. (1993), various strains of propionibacteria were tested for their ability to produce propionic acid on substrates such as maltose, lactose, lactate and starch. Based on the results obtained from the tests on the substrates, it was found that *Propionibacterium acidipropionici* produced the most propionic acid on lactate and that 14 g/L biomas in 140 h were produced when tested using a partially hydrolyzed corn substrate in batch fermentors. Lactate was the preferred substrate over lactose and glucose for propionic acid production by *P. acidipropionici* using continuous, immobilized cell bioreactors in studies done by Lewis and Yang (1992). Lactate fermentation yielded higher propionic acid concentrations, lower cell yields and lower specific growth rate.

Rehberger and Glatz (1998) screened seventeen strains for acid production and final pH on glucose, fructose, and maltose as the primary carbon sources. *Propionibacterium acidipropionici* was found to produce more acid and reached a lower final pH than any of the other strains. When tested for its ability to grow and survive at low pH with lactic, hydrochloric or propionic acid, propionic acid was found to be the most inhibitory. *P. acidipropionici* was not able to initiate growth and survive at lower pH values. No growth could be detected for strains at a pH below 5.0. Nevertheless, when started at neutral pH, the final pH reached values less than 4.4. Strains could grow at neutral pH in the presence of increasing lactate and propionate concentrations (180 m*M* and 150 m*M*, respectively). Hsu and Yang (1991) reported that *P. acidipropionici*

cultures are extremely pH-dependent. Quesada-Chanto et al. (1994) reported that an increase in the pH from 5.5 to 6.5 doubled the amount of propionic acid produced in a continuous culture with *P. acidipropionici* and that the optimum pH value for the production of propionic acid is between 6.5 and 6.8.

Quesada-Chanto et al. (1994) reported the optimum temperature for propionic acid production to be 37 °C. They arrived at this conclusion after observing a biomass increase and product concentration at temperatures ranging from 25 °C to 37 °C and a decrease in cell concentration when *P. acidipropionici* was incubated at 40 °C or higher in continuous culture.

According to Piveteau (1999), certain propionibacteria can utilize L-lactate preferentially over D-lactate when mixed together independent of the pH, initial lactate concentration and initial ratio of the two isomers. He explained the utilization of one isomer over the other by the fact that L-lactate metabolism increases the intracellular concentration of pyruvate thus inhibiting D-lactate dehydrogenase activity. Crow (1986) reported a 75% decrease in D-lactate dehydrogenase when tested against 20 mmol $\cdot$ L^{-1} pyruvate and 10 mmol $\cdot$ L^{-1} lactate was used as the substrate. Nevertheless, when 40 mmol $\cdot$ L^{-1} pyruvate was used, it decreased both L-lactate dehydrogenase and D-lactate dehydrogenase by 6 and 81, respectively. These tests were performed in *P. freudenreichii* subsp. *shermanii*, but similar trends have also been observed with *P. freudenreichii* subsp. *freudenreichii* and *P. acidipropionici* (Piveteau, 1999).

Interactions between propionic and lactic acid producing bacteria

Piveteau et al. (1995), studied the interactions between lactic acid and propionic acid producing bacteria. In their studies, *Lactobacillus helveticus* and *Streptococcus*

thermophilus were able to stimulate the growth of *P. freudenreichii* and *P. acidipropionici* with the former being the most consistent. In this same study, *L. helveticus* and *S. thermophilus* were chosen for a more detailed study of growth stimulation. It was shown that the increase in growth coincided with an increase in the conversion of lactate to propionate and acetate and that the lactate isomers behaved as described by Crow (1986). In contrast, Perez-Chaia et al. (1994), determined that *P. acidipropionici* exhibited a slow growth rate when grown with *Lactobacillus helveticus.* It was concluded the slow growth rate was due to the fast reduction of pH by *Lactobacillus* as it has been known to cease the growth of *P. acidipropionici* (Hettinga and Reinbold, 1972). For example, mixed-culturing *P. acidipropionici* and *P. freudenreichii* with two strains of *L. helveticus*, caused an inhibition in growth (Perez-Chaia et al.,1995). However, a third strain of *L. helveticus* was able to stimulate growth of *P. freudenreichii* as it's pH was shown to be the highest (> 4.5). Because this study involved the use of different strains of lactobacilli and propionibacteria, it was determined that the strains were cause in the behaviors and interactions observed between these microorganisms. Lee et al. (1976) showed similar interactions between *L. plantarum* and *P. shermanii.* They reported an inhibition in the growth of *P. shermanii* at a pH of (4.9) due to the high acid produced by *L. plantarum* when grown in a glucose-lactate mixture. The preference of lactate over glucose by propionibacteria was evidenced in this study, as there was a slight decrease in lactate concentrations at the beginning of the experiment; although not enough to overcome its inhibition. These results are in contrast to studies by Liu and Moon (1982) and Parker and Moon (1982), where *P. shermanii* was able to use the lactate produced by *L. acidophilus* at a high rate therefore, preventing the

accumulation of lactate in the medium; propionibacteria were therefore stimulated by mixed culturing.

Propionic acid and industrial uses

Propionic acid has many commercial uses. The acid and its salts are used in making plastics (ie. moulding plastics and textiles), fruit flavors (citronellyl propionate and geranyl propionate), perfume bases, herbicides and butyl rubber (Boyaval and Corre, 1995). It is also used in animal feeds, grain preservation, antifungal agents (calcium and sodium salts) (Gu et al., 1999) and has therapeutic properties such as in the use of sodium propionate in treating wound infections, arthritis, dermatoses (Boyaval and Corre, 1995) and in vitamin B_{12} production (Hettinga and Reinbold, 1972). In the food industry, it is used to prevent the growth of mold and ropiness in breads, cheeses, meats, fruits, vegetables, tobacco, grain and in preventing the blowing of canned frankfurter without causing an alteration in flavor, dipping caps, containers and wrappers in propionic acid solutions has also proved to be effective (Boyaval and Corre, 1995). Propionic acid, has nevertheless, been accepted as a safe product for consumers as it has been summarized as a Generally Recognized As Safe (GRAS) food and feed additive by the Food and Drug Administration (FDA) (Boyaval and Corre, 1995).

The U.S. is the main producer of propionic acid with 50,000 tons produced in 1982 at a cost of \$0.73 kg^{-1} and \$1.00 kg^{-1} in 1992 (Herrero, 1983;Boyaval and Corre, 1995). Nevertheless, the production of propionic acid must overcome several barriers in order to maximize its commercial efficiency and marketability. According to Gu et al. (1999), these barriers are: increasing the rate at which propionic acid is produced as a typical fermentation takes 3 days to reach only 20 g/L with a yield of less than 60%;

controlling end-product inhibition as it decreases the amount of propionic acid produced during fermentations (Herrero, 1983; Woskow and Glatz, 1991); and decreasing the costs involved in the separation of the acid due to its low concentration (< 60 g/L propionic acid) from acetic acid (a byproduct of the fermentation).

Several studies have been conducted in the ever increasing effort to maximize the production of propionic acid. *P. acidipropionici* was tested for its ability to produce propionic acid while immobilized in calcium alginate beads (Paik and Glatz, 1994). The fermentation substrates were corn steep liquor and a semi-defined laboratory medium in batch, fed-batch, and continuous fermentation. The cell density of the beads was 9.8×10^9 cells/g (wet weight). It was found that complete substrate consumption of glucose (in laboratory media) and lactate (in corn steep liquor) and maximum acid production occurred in 36 h in batch culture. This was much more than seen in free-cell fermentations. Maximum propionic acid concentrations in fed-batch fermentations were 45.6 g/L in corn steep liquor and 57g/L in the semidefined medium. According to the authors, the combination of rapid initiation of acid production with high accumulated levels of product acid makes fed-batch fermentation with immobilized cells ideal for propionic acid production. Continuous fermentations also fared well as high volumetric productivities of propionic acid ($0.96\ g\ L^{-1}\ h^{-1}$) were reported; much more than the authors had observed with free cells. Gu et al (1999) also used immobilized cell to increase propionic acid productivity as well as extractive fermentation to reduce the inhibition of propionic acid production by end-product. They reported the productivities of propionic and acetic acids at $0.46\ g/L^{-1}\ h^{-1}$ and $0.12\ g/L^{-1}\ h^{-1}$, respectively and an improved performance with extractive fermentation at a concentration of $13\ g/L^{-1}$. Schuppert et al. (1992), used a

three-electrode poised-potential system with cobalt sepulchrate as an artificial donor to study its effect on the growth and production of propionate in *P. acidipropionici.* This method allowed for the exclusive production of propionic acid without the production of acetic acid from the acid whey substrate and in continuous culture, propionate was formed as the only fermentation product up to a dilution rate of 0.04 h^{-1}. The authors suggested that even though productivities and growth yields were low, obtaining propionate as the only fermentation product was advantageous as the separation of end-products produced during propionate fermentations is difficult and costly.

Bacteriocins of propionibacteria

Bacteriocins are antimicrobial substances produced by certain bacterial species (Lyon et al., 1993). They have also been described as protein-containing molecules that exert a bactericidal action on susceptible bacteria (Tagg, et al., 1976). An excellent review on bacteriocins is available and has been written by Barefoot and Nettles (1993). Only two bacteriocins in the cutaneous propionibacteria classification have been identified as belonging to *P. acnes* (Fujimura and Nakamura, 1978; Paul and Booth, 1988) and a total of three in the classical propionibacteria produce by *P. jensenii* and *P. thoenii* (Grinstead and Barefoot, 1992; Lyon and Glatz, 1993; Ratnam et al., 1999).

Lyon and Glatz (1991) reported a bacteriocin produced by *P. thoenii* P127. It was shown to exhibit broad activity towards related species (*P. thoenii, P. jensenii,* and *P. acidipropionici*) as well as possessed activity against gram-negative and gram positive bacteria, molds and yeasts. When tested on psychrotrophic spoilage or pathogenic organisms such as: *Lysteria monocytogenes, Pseudomonas flourescens, Vibrio parahaemolyticus, Yersinia enterocolitica,* and a *Corynebacterium* sp., strains were

inhibited when grown in skim milk fermented by *P. thoenii* P127. This was evidenced by a loss in number of viable cells after 24 h at 10 °C. The uniqueness of this bacteriocin is in its activity against gram-negative bacteria (Barefoot and Nettles, 1993). This bacteriocin, named Propionicin PLG-1,was isolated and purified in studies done by Lyon and Glatz (1993). In these studies, isolation was made possible by allowing cultures to grow to a late stationary phase then analyzing the supernatant for propionicin PLG-1. Maximum production of propionicin from *P. thoenii* P127 was obtained at pH of 7.0 after anaerobic incubation for 180 h in sodium lactate broth. At a pH of 6.0, 6.5 and 7.5, and 8.0, bacteriocin production declined. Purification with ammonium sulfate ion-exchange chromatography and isoelectric focusing revealed a protein band with a molecular weight of 10,000-Da.

Grinstead and Bearfoot (1992) reported on the first heat-stable bacteriocin produced by the diary or classical propionibacteria. Jensenii G, is the bacteriocin produced by *P. jensenii* P126. Its molecular size is 12,000-Da and has demonstrated heat-stability at 100 °C for 15 minutes. This is in contrast to propionicin PLG-1, which is heat liable at 85 °C (Lyon and Glatz, 1991). The above mentioned investigators found Jensenii G to exhibit a narrow spectrum of activity towards related propionibacteria, lactococci and lactobacilli. This bacteriocin showed a bactericidal rather than a bacteriolytic action on *P. acidipropionic* as the addition crude jensenii G to 1.9×10^7 cells of the propionibacteria strain caused in total cessation of growth but not cell death. Lower levels of jensenii G (5 to 10 AU/mL) had no effect on growth of *P. acidipropionici* P5. It was also found that jensenii G has the ability to inhibit dairy lactobacilli. This was evident as viable cell numbers of *L. delbrueckii* subsp. *lactis* 4797 were reduced by 99%, indicating a

bactericidal action against the lactobacilli. The reason why jensenii G inhibits dairy lactobacilli more that propionibacteria is not known. Although they have suggested the availability of more receptors on the surface of *L. delbrueckii* subsp. *lactis* 4797 for jensenii G than on *P. acidipropionici* P5.

After screening thirteen propionibacteria strains, eight were selected because of their production of protease-sensitive, catalase-insensitive agents (bacteriocins) which caused the inhibition of closely related species of propionibacteria, lactobacilli and lactococcus (Ratnam et al., 1999). Zones of inhibition were observed in *P. jensenii* B1264 and was found to produce the broadest inhibitory spectrum. It was chosen for further studies as the mode of action for this bacteriocin revealed inhibitory activity during late stationary phase toward *L. delbrueckii* subsp. *lactis* ATCC 4797 producing a 90% loss in viability within 60 minutes. The purification of the inhibiting substance was reported to have an estimated molecular mass of 6 to 9 kDa. It was shown to hold its stability when heated at 100 °C for 60 minutes which was much in contrast to jenseniin G exhibiting stability at 100 °C for 15 minutes (Grinstead and Barefoot, 1993) and PLG-1 showing a heat stability of ≤85 °C (Lyon and Glatz, 1991). A wide range of tolerance towards pH was reported ranging from 3.0 - 12.0; a much wider range than reported for PLG-1 (3.0 - 9.0) (Lyon and Glatz, 1991) and a similarity towards jenseniin G at 3.0 - 12.0 (unpublished results; Ratnam et al., 1999). Dissimilarities were observed from the spectrum activities of propionicin PLG-1 and jenseniin G. This antagonist inhibited *P. thoenii* P127 and showed no action towards gram-negative bacteria (unlike propionicin PLG-1). In contrast to the results observed in jenseniin G activity (Grinstead and

Bearfoot, 1992), the antagonist inhibited the producer of jenseniin G, *P. jensenii* P126, as well as *L. acidophilus* ATCC 4356 and *L.* casei ATCC 7469.

Propionibacteria and probiotics

Much of the work done on probiotics has been on lactic acid and bifidobacteria (Mantere-Alhonen, 1995; Fuller, 1997) extending to the yeast and fungal organisms utilized in cattle (Huber, 1997). Probiotics are bacteria that promote health towards humans and animals (Mantere-Alhonen, 1995). Criteria have been established for the ideal probiotic. These include the ability for the microorganisms to maintain viability during processing and storage, demonstrate a resistance to adverse changes in pH, show adherence to intestinal epithelial cells, and maintain viability and high cell concentration during passage through the intestine (Mantere-Alhonen, 1995;Gibson, et al., 1997).

Propionibacteria have been evaluated for their effects on stimulating growth in animals. Antipov and Subbotin (1980; as cited by Mantere-Alhonen, 1995) combined propionibacteria with various lactobacilli and bifidobacteria species for ameliorating disorders of the digestive tract in calves, piglets and chickens with positive results. In these trials, the bacterial concentration used was 4.0 to 6.0 x 10^9 cfu/g. This is much in agreement with Raibaud (1992) as he demonstrated that bacteria introduced into the gut need to be at concentrations between 5 x10^8 to 1 x10^{11} in order to play a role in the gastrointestinal ecosystem. Other work by Cerna et al. (1991) showed a reduced daily feed intake and increased daily weight gain in calves fed a preparation called Proma. Proma is a preparation of *L. plantarum*, Enterococcus *faecium*, *Lactococcu lactis* including the propionibacterium, *P. freudenreichii*. The concentration used in this study was 2 x 10^8 cfu/g; again in agreement with Raibaud (1992). After withdrawal of a diet

supplemented with Proma, calves continued to show an increase in weight of 11.3 to 21.1 % faster than the control group. Tests with piglets fed different species of propionibacteria were investigated and *P. freudenreichii* ssp. *shermanii* was found to exert the most effective probiotic properties (Mantere-Alhonen, 1982 [as cited by Mantere-Alhonen, 1995). Mantere-Alhonen (1995) reports this as being the first study to use pure propionibacteria in regards to its effect as a probiotic and a growth promoter. In this study, 230 piglets were given 1 to 5 g/d per animal of *P. freudenreichii* in their feed. The concentration was 2 x 10^9 cfu/g. The treated groups had higher weight gains (9.2 to 14.5 %) and lower feed intakes (7.2 to 46.1 %) than control groups and reduction in diarrhea was also observed. It was concluded that even though no colonization of the propionibacteria was evident in the intestines of the piglets, the positive results obtained were an indication of the benefits in utilizing propionibacteria as probiotic. As mentioned before, an important characteristic for a probiotics is its tolerance to the various interactions taking place in the gastro-intestinal tract of animals or humans. Mantere Alhonen (1983), tested the survivability of *P. freudenreichii* during an in-vitro gastric digestion. In this study, the culture was treated with hydrochloric acid (0.15 N), rennet (2.1%), and pepsin (1%) and incubated at 37.2 °C in a water bath supplemented with a shaker to simulate the peristaltic movements of the gastro-intestinal tract. After 6 h of incubation, colony counts revealed no effect on the viability of the microorganism as counts remained steady at a log of 10^7 cfu/mL in spite of the decreasing pH from 6.7 to a final of 4.8.

Perez-Chaia et al. (1995) evaluated the effects of a dairy propionibacteria and it's establishment in the gut. The strain used in this study was a *P. acidipropionici* CRL 1198

isolated from Emmental cheese. The approach towards determining the survival or establishment in the gut was to feed mice a mixture of skim milk containing 10^8 bacteria/mL for 7 days and determine the population of the surviving microorganism by fecal sampling. Results revealed an increase of 10^{10} propionibacteria per gram and a week after the cessation of the diet, the amounts of propionibacteria were reduced in the large intestine but not in the small bowel. Perez-Chaia et al (1995) suggested the persistence was due to adhesion factors in *P. acidipropionici.* Mukai et al. (1994) suggested external components on the cell wall of bacteria may contribute to the adhesion of the intestinal mucosa. Perez-Chaia et al. (1995) found that *P. acidipropionici* CRL 1198 possesses the ability to reverse the hyperlipemic effect of a diet with a high fat content. Mice fed cream supplemented with 10^8 propionibacteria /mL, showed lower serum lipid concentration than the group fed the cream alone. These authors postulated that a lipid-lowering effect exists in propionibacteria when given with milk.

Perez-Chaia et al. (1999) studied the potential probiotic properties of propionibacteria in their ability to decrease fecal enzymes possessing the capacity to convert pro-carcinogens into carcinogens. Red cooked meat, which induces carcinogenic compounds (Mallett et al., 1983) was fed to mice which received skim milk supplemented with 5 x 10^8 cell/day, skim milk or water for 14 d. The diet supplemented with *P. acidipropionic* CRL 1198, was found to prevent the activity of β-glucuronidase caused by the red meat diet on the 7^{th} day of feeding when *P. acidipropionic* was found at 7 x 10^9 cfu per gram of feces. The lowest level of β-glucuronidase also coincided on the 7^{th} day of feeding. Workers concluded that propionibacteria may play a role in preventing colon cancer.

Isolation and characterization of *Propionibacterium acidipropionici* DH42

Dawson et al. (1994) isolated a bacterium from ensiled, high-moisture corn that produces propionic acid and acetic acid from glucose or lactate. It was described by workers as a gram-positive, facultative anaerobe, non-sporeforming, nonmotile, pleiomorphic rod which formed clumps when grown in peptone-yeast extract-lactate medium. The colonies were described as pin-point, 1mm in size, yellow in color, circular and possessed a catalase reaction when grown aerobically for five days at 30 °C. This bacterium produced propionate and acetate from lactate and glucose. Under anaerobic conditions, the bacterium grows rapidly utilizing glucose in liquid media. Anaerobically, the bacteria tends to grow slowly on solid media. Maximum growth peaked after 12 to 24 h on .5X MRS incubated at 30 °C and terminal pH after 48 h on purple base-glucose and .5X MRS was 3.9 and 4.1

The bacterium was given the name *Propionibacterium acidipropionici* DH42 as the phenotypic characteristics of this bacterium were much like that of the ATCC type strain *Propionibacterium acidipropionici.* In a review of the literature by the authors, no published studies were found regarding the isolation of a propionibacterium from ensiled feeds.

RESEARCH OBJECTIVES

The goal of this research was to determine the ability of *Propionibacterium acidipropionici* DH42 in preventing the accumulation of lactic acid during in-vitro fermentations and during experimentally induced acidosis in ruminally fistulated cattle.

SPECIFIC OBJECTIVES

1. To test the effectiveness of *P. acidipropionici* DH42 in reducing the lactic acid levels produced by in-vitro fermentations with wheat.
2. To determine the probiotic effects of *P. acidipropionici* DH42 in its ability to prevent reductions in pH and increases in lactic acid associated with lactic acidosis syndrome during experimentally induced lactic acidosis in cattle.

MATERIALS AND METHODS

In-Vitro Studies

Preliminary studies on effectiveness of media. One and a half percent agar (Difco, Detroit, MI) was added to a mixture of Purple Broth Base (BBL 11558 Cockeysville, MD) with 1% erythritol (Sigma Chemical Co. St. Louis, MO) were used as the general media in studies assessing its use as a selective and differential media for the enumeration of *P. acidipropionici* DH42 cells in pure culture, in a mixed culture (rumen fluid) and compared against a control (rumen fluid without addition of *P. acidipropionici* DH42). Rumen fluid utilized was from a dairy cow or a beef steer. One gram of freeze-dried *P. acidipropionici* DH42 obtained from Laporte Biochem International, Milwakee, WI, was used as the inoculant. Fermentors were incubated at 38 °C and sub-samples were collected every 6 or 8 h for a total of 24 h. Cell recoverability was determined by the spread plate technique with appropriate dilutions in duplicated plates. Plates were placed in glass dessicators, gassed with CO_2 for one minute, and incubated anaerobically for 5 days at 38 °C. Yellow colonies were counted and the effectiveness of media was determined as the number of colony forming units per 1 mL of sub-sample.

Fermentor studies and experimental treatments. Rumen fluid was collected from a steer at the Beef Cattle Research and Teaching Center which was maintained on a high concentrate diet, strained through four layers of cheese-cloth, and transported to the laboratory. A total volume of 500 mL, consisting of 250 mL freshly strained rumen fluid and 250 mL of McDougall's buffer was added to each of five dual-port fermentors with built in magnetic stir rods (Microcarrier Spinner Flask, Model #1965, Belco Glass, Inc., Vineland, NJ). Wheat, ground through a 1mm screen (Cyclotec, Tecator Inc., Herndon,

VA) was added at 2% of the volume of each fermentor and served as the carbohydrate source. Additions to the fermentors were 1 mL distilled water (Control), 1 mL of *P. acidipropionici* DH42 at total amounts of: 10^9 cfu (F1), 10^8 cfu(F2), 10^7 cfu(F3), and 10^6 cfu (F4). *P. acidipropionici* DH42 was available in a commercial freeze-dried form (Laporte Biochem International, Milwakee, WI). The cells in the packet were enumerated for total viable numbers by using the spread plate technique before initiation of the experiments. One gram was added to 500 mL peptone (Difco, Detroit, MI), serially diluted, and plated in duplicates. Duplicated plates were incubated anaerobically at 38 C for 5 days before counting colonies. Viability yielded 2.4×10^{11} cfu/g. The media used to evaluate the viability of the freeze dried *P. acidipropionici* DH42 was Reinforced Clostridial Medium (OXOID, Basingstoke, Hampshire, UK). Purple Base Broth (BBL 11558, Cockeysville, MD) supplemented with 1.5% agar (Difco, Detroit, MI) and 1% erythritol (Sigma Chemical Co., St. Louis, MO) was used to enumerate the numbers of *P. acidipropionici* DH42 during the fermentation studies. Incubation was as described above.

Sampling schedule and analytical procedures. The fermentors were gassed for one minute with CO_2, sealed tightly, and incubated at 38 °C on stir plates. The incubation was for a total of two hours after the addition of the ground wheat to allow its fermentation to proceed. The addition of DH42 was called time zero which initiated the sampling time frames. Five mL of fermentor media was sampled every eight hours starting with time zero, for a total sampling period of 48 h. Fermentors were re-gassed, resealed and re-incubated at each sampling. At the end of the first 24 h, 10^{10} cfu of DH42 were added to all fermentors excluding the control. Sampling was continued as described for

the remainder of the 40 h. All samples were analyzed for pH, cell viability, and volatile fatty acid determination. One mL aliquots were removed for viable cell counts as described above and the remaining 4 mL were acidified with 1 mL of a 12 N solution of sulfuric acid and frozen for later analysis of volatile fatty acids and lactic acid. The one mL aliquots for microbiological analysis were plated in duplicates in their appropriate dilutions. Plates were incubated anaerobically at 38 °C for 5 days before counting colonies. Fermentation studies were conducted on two different days following the same procedure as described above as to obtain duplicated results.

HPLC analysis of fermentation end-products. Lactic acid and volatile fatty acids were quantified by ion-exchange-exclusion HPLC (BIORAD aminex HPX-87H, Richmond, CA). The mobile phase consisted of .005 N H_2SO_4 at a flow rate of .6 mL/min. Column temperature was regulated by an external column heater (Waters Millipore, Milford, MA) at 65 °C. Four mL of fermentor samples were centrifuged for 30 minutes at 26,000 x g and placed into 3 mL HPLC sample vials (National Scientific, Atlanta, GA). Centrifuged samples were placed at 4 °C until analyzed. Fifteen mL of the centrifuged samples were injected by an autoinjector (Waters WISP 712, Milford, MA) and analytes were detected by refractive index (Waters 410 refractive index detector, Milford, MA). Peak heights of individual volatile fatty acids were quantified by a commercial HPLC software package (Turbochrom 3, PE Nelson, Cupertino, CA) and compared to a mixed standard solution containing lactic acid and volatile fatty acids (Supelco, Bellefonte, PA).

Statistical analysis. Data were analyzed using the mixed model analysis (Proc Mixed) of SAS (1997), with the random effect of treatment nested within the experimental

replication. The least squares means were compared using the Tukey-Kramer procedure for multiple comparisons. Fermentor pH, lactic acid, volatile fatty acids, total volatile fatty acids, and cell recoverability were analyzed using a fixed classification model with interactions among levels of main factors. The model was:

$$y_{ijk} = \mu_{ijk} + P_j + T_k + PTr_{jk} + e_{ijk}$$

where:

y_{ijk} is the variable measure (fermentor pH, lactic acid, propionic acid, acetic acid, butyric acid, valeric acid, isobutyric acid and isovaleric acid concentrations, total volatile fatty acids, and cell recoverability) of fermentor (i) containing bacterial dose (j) sampled after (k) hours;

μ_{ijk} is the overall mean;

P_j is the fixed effect of the sampling period (Six levels: 0, 8, 16, 24, 32, 40, and hours);

T_k is the fixed effect of the treatment (Five levels: 10^9 cfu, 10^8 cfu, 10^7 cfu, 10^6 cfu, 10^9 cfu);

PT_{jk} is the two-way interaction of the sampling period and treatment ;

e_{ijk} is the random residual effect pertaining to every record.

In-Vivo Studies

Preliminary animal studies. Four rumen fistulated steers fed a high concentrate diet composed of dry rolled corn and corn silage (80:20) were used to determine the amount of wheat necessary to provoke experimental acidosis. The steers were maintained a the Beef Cattle Research Teaching Center Metabolism Room. Four wheat slurry dose levels were used and each dose corresponded to an individual animal. Ground wheat was used as a slurry by adding water in the ratio of 1 part wheat: 2 parts warm water (weight/volume) as follows: 10 g/kg BW, 20 g/kg BW, 30 g/kg BW and 40 g/kg BW.

The wheat slurry was prepared by hand mixing appropriate amount of wheat, as calculated by the animal's body weight, in large plastic tubs with warm tap water (30 to 35 °C) as monitored using a hand held thermometer. Wheat slurry was then poured into the rumen via the rumen fistula of each animal. Samples were collected every 2 h for 10 h and rumen pH was recorded. Rumen contents were evacuated when ruminal pH approached 5.0 as to prevent irreversible acidosis. Evacuation of ruminal contents was performed by diluting the rumen contents with warm water (30 to 35 °C) and then siphoning contents out with a 5 cm diameter plastic hose. This was repeated until rumen was empty. Animals were reinoculated with 2 liters of rumen contents from a healthy donor steer and hay was offered. The following morning, animals were taken out of the Metabolism Room and placed in large holding pens and allowed free roaming access.

Animals, diet and experimental treatments. Six rumen fistulated steers in a two-period crossover design weighing 500 to 550 kg were provided a diet of dry rolled corn and corn silage (80:20) prior to the trial. The steers were fed once a day in the morning. Approval to perform the animal studies was granted by the All-University Committee on Animal Use and Care (# 11/95-136-00). Three animals were treated with 10^{11} cfu as determined by plating for viability of the freeze-dried DH42 utilizing the same method as in the in-vitro studies. Viability assessed before each trial. *P. acidipropionici* DH42 was administered via rumen fistula for 2 weeks before the initiation of the experimental period and the other three were given no DH42 and served as the controls. Propionibacteria in this study was available as a freeze-dried commerical product from Laporte Biochem International, Milwakee, WI. The treatment sequence involved 2 periods, and the interval between period 1 and period 2 was two weeks. Animals serving

as controls during the first period were dosed with DH42 in period 2 and vice versa. Acidosis was induced by the intraruminal administration of a slurry of 1 part finely ground wheat to 2 parts warm tap water forming a dose of 40 g/kg BW as determined by preliminary animal studies. Animals were fasted 24 h prior to the feeding of the slurry. Freeze-dried *P. acidipropionici* DH42 was reconstituted in 100 mL of warm tap water (30 °C), swirled gently, and immediately poured into the rumen via the rumen fistula, 1 h before the morning feeding. Cattle were not fed for 24 h before the carbohydrate overload, but allowed free access to water. The last dose of DH42 was given with the last morning feeding, approximately 24 h before the acidosis challenge.

The end of the test period was determined to be when rumen pH values fell below 5.0 at which time the rumen contents were totally evacuated, washed with warm water, and re-inoculated with fresh rumen contents from a healthy donor steer receiving control diet. Steers were then given hay and released from their pens the next morning, at which time they resumed eating a dry corn and corn silage diet as described above.

Sampling schedule and analytical procedures. Rumen fluid and jugular blood samples were obtained from each steer before and at 2, 4, 6, 8, and 10 h (end of test period as determined by rumen pH) after the initial carbohydrate dosing. Jugular catheters were placed in all steers before the initiation of the experimental period and flushed regularly with a sterile heparinized solution (200 U/mL heparin, .9 % NaCl, 1 % benzyl alcohol). Rumen contents were hand mixed thoroughly and sampled at random in three different sites. Rumen fluid pH was recorded immediately after collection and strained through 4 layers of cheesecloth. Two 30 mL aliquots samples of rumen fluid were collected for enumeration of total numbers of *P. acidipropionici* DH42 and for the

analysis of lactic and volatile fatty acids. Samples collected for acid analysis were treated with 1 mL 12 N sulfuric acid and were frozen for later analysis by HPLC. Jugular blood for blood pH analysis and blood lactic acid concentrations were collected with a heparinized syringe connected to flouride/oxalate vacutainers. Blood samples were immediately placed on ice and analyzed within 30 minutes after collection for pH determination using a blood gas analyzer (Stat 4 Profile, Nova Biomedicals). Samples were centrifuged and blood plasma was collected and stored frozen for later analysis. Blood plasma L(+) lactate was determined by an enzymatic method after being deproteinized with 10 % trichloroacetic acid (Sigma Diagnostic Kit 826-B, Sigma Chemical, St. Louis, MO).

Microbiological procedures and media. Ruminal fluid samples were collected at the time frames as described above for the determination of the recoverable numbers of *P. acidipropionici* DH42 during ruminal acidosis. Rumen fluid was plated on duplicate spread plates with their appropriate dilutions. Plates were place in glass dessicators, flushed with CO_2, and incubated anaerobically for 5 days at 38 °C. At the end of 5 days, colonies were counted and the recoverable numbers of *P. acidipropionici* DH42 were determined. The medium used was as described above in the in-vitro studies in which purple broth base (BBL 11558, Cockysville, MD), 1% erythritol (Sigma Chemical Co., St. Louis, MO), and 1.5% agar (Difco, Detroit, MI) were used. Peptone broth (Difco, Detroit, MI) was used for the serial dilutions. Samples were immediately transported to the laboratory where they were analyzed within 30 minutes after collection.

HPLC analysis of fermentation end-products and total blood lactic acid determination. Rumen lactic acid and VFA were analyzed utilizing the method described

in the in-vitro fermentation studies. Total blood lactate was analyzed using the same method as described for the determination of lactic acid and volatile fatty acids in rumen fluid with the exception that total plasma lactate was detected by reversing the polarity on the refractive index (Waters 410 refractive index detector, Milford, MA). D (-) lactate was obtained as the difference between the total blood lactate as analyzed by the HPLC method and L(+) lactate as analyzed enzymatically.

Statistical analysis. Data were analyzed using the mixed model analysis procedure (Proc Mixed) of SAS (1997) with animal used as a random effect. The least square means were compared using the Tukey-Kramer procedure for multiple comparisons. A fixed classification model with interactions among levels of main factors was applied to data of rumen pH, blood pH, blood lactates, rumen lactic acid, rumen volatile fatty acids, rumen total volatile fatty acids, and dry matter intake (lb/d). The model equation was:

$$y_{ijkl} = \mu_{ijkl} + T_j + P_l + Tr_k + TP_{jl} + TrP_{kl} + TrT_{kj} + e_{ijkl}$$

where:

y_{ijkl} is the variable measured (rumen pH, blood pH, blood lactate, rumen lactic acid, propionic acid, acetic acid, butyric acid and succinic acid, total rumen volatile fatty acids, and dry matter intake) of animal (i) belonging to group (j) during trial (k) sampled after (l) hours;

μ_{ijkl} is the overall mean;

T_j is the fixed effect of treatment (two levels; control and treated);

P_l is the fixed effect of the period of sampling (six levels: 0, 2, 4, 6, 8, 10 hours);

Tr_k is the fixed effect of the trial (two levels: Trial A and Trial B);

TP_{jl} is the two-way interaction of treatment and period of sampling;

TrP_{kl} is the two-way interaction of trial and period of sampling;

TrT_{kj} is the two-way interaction of trial and treatment;

e_{ijkl} is the random residual effect pertaining to every value.

RESULTS AND DISCUSSION

In-vitro Studies

In pure cultures, *P. acidipropionici* has the ability to utilize various carbon sources, specifically, glucose and lactate (Lewis and Yang, 1992). However, *P. acidipropionici* will use lactic acid preferentially when both glucose and lactic acid are present (Lee et al., 1974). Because of these abilities, inoculation of *P. acidipropionici* DH42 into simulated ruminal fermentations would be beneficial in preventing the accumulation of lactate. In the present study, four treated and one control fermentor were filled with rumen fluid from a steer adapted to a high-concentrate diet. Fermentors included the following doses: Control (no DH42), Trt 1 (10^9 cfu), Trt 2 (10^8 cfu), Trt 3 (10^7 cfu) and Trt 4 (10^6 cfu). All the fermentors contained doses that were either at or well below the numbers of naturally occurring lactate-utilizing bacteria (approx. 10^9) found in the rumen of high-concentrate fed animals (Mackie and Gilchrist, 1979). It should be noted that the low values for lactic and volatile fatty acids observed in the controls at 0 h in the current study, are a direct consequence of missing data due to human error (see Appendix A).

In this study, treatment had no effect ($P > .05$) on the pH of the rumen fluid (Table 1) in our fermentors. A decrease ($P > .05$) in pH was observed from 0 to 8 h in all fermentors and remained virtually constant with no further decreases throughout the remainder of the sampling period. It is known that the feeding of carbohydrates causes changes in the microbial population, its fermentation patterns, and in the decline of rumen pH parallel to increases in lactic acid (Mackie and Gilchrist, 1978; Slyter and

Table 1. The effect of the addition of *P. acidipropionici* DH42 at different dose levels on rumen fluid pH during in-vitro fermentations.

	Treatment Dose, cfu					
Rumen Fluid, pH						
Time (h)	0 (control)	10^9	10^8	10^7	10^6	SEM
0	5.98	5.99	6.00	5.91	5.92	0.29
8	5.55	5.51	5.50	5.35	5.34	
16	5.51	5.49	5.48	5.38	5.32	
24	5.54	5.51	5.51	5.38	5.34	
32	5.54	5.50	5.50	5.38	5.34	
40	5.56	5.53	5.53	5.40	5.38	

Means are averages of 2 replications

Rumsey, 1991). It can be suggested that because no significant reduction ($P > .05$) in ruminal fluid pH was observed in any of the fermentors, the lactate-utilizing microbial population of the rumen were possibly able to rapidly metabolize lactic acid as fast as it was being produced. This could be a possibility because the ruminal fluid initially collected for use in the current fermentation study was of a steer adapted to a high grain diet. Mackie and Gilchrist (1978) have found elevated ruminal pH levels ($pH > 5.78$) in sheep during a stepwise adaptation to a high concentrate diet and attributed it to the low levels of lactic acid found in their experiments. However, fermentors treated with *M. elsdenii*, a lactate-utilizer, exhibited higher ruminal pH after stabilizing at 5.4 during the entire study than the control cultures which decreased to 4.8 for the remainder of the 24 h fermentation period (Kung and Hession, 1995).

Lactic acid was not produced in any of the fermentors as evidenced by HPLC analysis or the quantities were too negligible (Table 2) to be detected. In addition, treatment effect was not significant ($P > .05$). There was however, a significant increase ($P < .05$) of lactic acid in the control culture at 16 h and then again at 40 h from 0 h and in the fermentor treated with 10^9 cfu DH42 at 16 h.

Table 2. The effect of the addition of *P. acidipropionici* DH42 at different dose levels on the production of lactic acid during in-vitro fermentations.

	Treatment Dose, cfu					
Lactic acid, m*M*						
Time (h)	0 (control)	10^9	10^8	10^7	10^6	SEM
0	$0.00^{b‡}$	0.00^{b}	0.00	0.00	0.00	0.004
8	0.00^{b}	0.00^{b}	0.00	0.00	0.00	
16	0.02^{a}	0.01^{a}	0.00	0.00	0.00	
24	0.00^{b}	0.00^{b}	0.00	0.00	0.00	
32	0.00^{b}	0.00^{b}	0.00	0.00	0.00	
40	0.02^{a}	0.00^{b}	0.00	0.00	0.00	

[ab]Means within a column with unlike superscripts are significantly different ($P < .05$)
Means are averages of 2 replications
‡SEM = .005 (due to missing data)

In fermentation studies by Kung and Hession (1995) with *M. elsdenii* and by Wiriyawan and Brooker (1995) with *M. esldenii* and *Selenomonas ruminantium*, lactic acid was always present in control cultures as a major end product of carbohydrate fermentation. The presence of lactic acid was expected as it is well known that adding a source of glucose or starch to ruminal contents under an anaerobic environment causes the proliferation of amylolytic or lactate-producing bacteria (Slyter, 1976; Mackie and Gilchrist, 1979). It is possible, however, that the microbial population of our rumen fluid was not affected by the addition of our carbohydrate source (10 g wheat/500 mL rumen fluid media) as the rumen fluid utilized in this study was of a steer adapted to a high-concentrate diet or that the level of the substrate offered might of been too low to elicit an effect. Low levels of lactic acid have been observed during step-wise adaptations to a high-concentrate diet in sheep (Mackie and Gilchrist, 1979).

The addition of *P. acidipropionici* DH42 to our fermentors at different inoculation rates caused no effect ($P > .05$) on the levels of propionic acid produced. A significant increase ($P < .05$) in the production of propionic acid was only observed from 0 to 8 h in the control fermentor (18.46 to 31.78 m*M*) and remained slightly unchanged throughout the end of the sampling period (Table 3). No significant increases were noted in the DH42 treated fermentors as the trend past 8 h was much like that of the control fermentor. In contrast to these results, Kung and Hession (1995) observed decreases ($P < .05$) in propionic acid production after initial increases up to 4 h during in-vitro fermentations with a ruminal lactate-utilizer, *M. elsdenii*, regardless of dose (8.7×10^5 cfu/mL or 8.7×10^6 cfu/mL) in treated cultures. Increases in propionic acid relative to the trace amounts of lactic acid found in the current study could reflect the active metabolism of lactic acid into propionic acid by lactate-utilizers.

Table 3. The effect of the addition of *P. acidipropionici* DH42 at different dose levels on the production of propionic acid during in-vitro fermentations .

	Treatment Dose, cfu					
Propionic acid, m*M*						
Time (h)	0 (control)	10^9	10^8	10^7	10^6	SEM
0	18.46[b‡]	25.42	24.75	25.50	24.64	7.04
8	31.78[a]	31.15	29.50	30.21	29.35	
16	31.60[a]	30.83	29.92	30.98	30.36	
24	29.05[a]	30.23	29.46	30.18	31.29	
32	28.42[a]	29.48	29.68	30.98	32.01	
40	30.60[a]	30.34	30.01	31.34	30.62	

[ab]Means within a column with unlike superscripts are significantly different ($P < .05$)
Means are averages of 2 replications
[‡]SEM = 7.71 (due to missing data)

No treatment effect ($P > .05$) was observed on the production of acetic acid (Table 4) in the control and treated fermentors. However, a non-significant increase ($P > .05$) in the production of acetic acid was noted from 0 to 8 h in all treated fermentors and significant increase ($P < .05$) was noted in the control fermentor from 0 to 8 h (43.22 to 81.76 m*M*). Again, levels remained constant throughout the end of the sampling period (40 h) in all fermentors. Kung and Hession (1995) observed a different pattern. They reported increases in acetic acid levels up to 4 h with decreases thereafter regardless of dose.

Treatment had no effect on ($P > .05$) the levels of butyric acid (Table 5) produced in this study. Within the treated fermentors, no significant differences ($P > .05$) were observed over time. However, a significant increase ($P < .05$) from 0 to 8 h (8.59 to 20.08 m*M*) was observed in the control fermentor and is a direct consequence of missing data as stated earlier. The main reason for this difference being that their choice of microorganism for use in their fermentation studies, *M. elsdenii,* is one of the main producers of butyric acid in the rumen (Slyter, 1976; Ogimoto and Imai, 1981).

Treating our fermentors with different doses of *P. acidipropionici* DH42, produced no effect ($P > .05$) on the levels of valeric acid (Table 6). Within the treated fermentors, significant increases ($P < .05$) in valeric acid were noted at 8 h. Past 16 h production in this acid leveled off as amounts remained practically unchanged. A significant increase ($P < .05$) in valeric acid was found at 16 h in the control fermentor and remained unchanged past 16 h as compared with the treated ferrmentors. In Kung and Hession's (1995) study, valeric acid levels were increased ($P < .05$)) in treated cultures as opposed to the controls.

Table 4. The effect of the addition of *P. acidipropionici* DH42 at different dose levels on the production of acetic acid during simulated in-vitro acidosis .

	Treatment Dose, cfu					
Acetic acid, m*M* Time (h)	0 (control)	10^9	10^8	10^7	10^6	SEM
0	38.57[b‡]	60.24	58.83	57.73	53.90	11.60
8	81.76[a]	81.01	79.24	74.07	72.01	
16	82.68[a]	80.88	82.15	76.22	76.00	
24	75.92[a]	80.20	81.01	74.83	75.77	
32	76.81[a]	79.45	79.97	77.50	77.80	
40	80.58[a]	79.96	81.42	77.67	74.92	

[ab]Means within a column with unlike superscripts are significantly different ($P < .05$)
Means are averages of 2 replications
‡SEM = 15.72 (due to missing data)

Table 5. The effect of the addition of *P. acidipropionici* DH42 at different dose levels on the production of butyric acid during in-vitro fermentations .

	Treatment Dose, cfu					
Butyric acid, m*M* Time (h)	0 (control)	10^9	10^8	10^7	10^6	SEM
0	8.59[b‡]	13.99	13.70	13.80	12.92	3.69
8	20.08[a]	19.99	19.04	17.92	17.44	
16	20.62[a]	20.60	20.19	18.66	18.41	
24	19.05[a]	20.32	20.04	18.10	18.85	
32	18.69[a]	19.88	20.15	18.71	19.25	
40	19.88[a]	20.31	20.60	19.96	18.86	

[ab]Means within a column with unlike superscripts are significantly different ($P < .05$)
Means are averages of 2 replications
‡SEM = 4.55 (due to missing data)

Table 6. The effect of the addition of *P. acidipropionici* DH42 at different dose levels on the production of valeric acid during in-vitro fermentations.

	Treatment Dose, cfu					
Valeric acid, m*M*						
Time (h)	0 (control)	10^9	10^8	10^7	10^6	SEM
0	2.01[b‡]	1.83[c]	1.77[c]	1.76[c]	1.61[d]	0.80
8	2.88[b]	2.86[b]	2.76[b]	2.61[b]	2.51[c]	
16	3.63[a]	3.59[a]	3.44[ab]	3.24[a]	3.10[bc]	
24	3.78[a]	3.91[a]	3.70[a]	3.44[a]	3.47[ab]	
32	3.60[a]	3.87[a]	3.72[a]	3.91[a]	3.91[a]	
40	4.01[a]	4.04[a]	3.88[a]	3.79[a]	3.83[a]	

[abcd] Means within a column with unlike superscripts are significantly different ($P < .05$)
Means are averages of 2 replications
‡SEM = 0.84 (due to missing data)

They suggested increases in valeric acid could be due to a substitution of propionyl-CoA for acetyl-CoA during the synthesis of fatty acids which would ultimately cause decreases in propionic acid levels (Marounek and Bentos, 1987).

Isobutyric (Table 7) and isovaleric (Table 8) acid levels were not affected ($P > .05$) by the addition of *P. acidipropionici* DH42. Fermentors dosed with 10^9 cfu and 10^8 cfu DH42, behaved much like the control fermentor as levels of isobutyric acid increased significantly ($P < .05$) at 16 h and remained slightly unchanged throughout the remainder of the experiment. Fermentors treated with 10^7 cfu and 10^6 cfu DH42 showed significant increases ($P < .05$) in isobutyric acid at 8 h. Kung and Hession's (1995) study reported a similar pattern in the production of isobutyric acid as compared to the controls of the current study, in which an initial concentration of approximately .78 m*M* as reported. However, lower concentrations of this acid were reported at 8 h (approximately 1.0 m*M*) as compared to the current study. Increases in isobutyric acids over time in fermentors treated with the high (8.7×10^6 cfu/mL) and low (8.7×10^5 cfu/mL) doses of *M. elsdenii*

Table 7. The effect of the addition of *P. acidipropionici* DH42 at different dose levels on the production of isobutyric acid during in-vitro fermentations.

	Treatment Dose, cfu					
Isobutyric acid, m*M*						
Time (h)	0 (control)	10^9	10^8	10^7	10^6	SEM
0	$0.87^{b‡}$	1.04^{c}	0.99^{c}	0.97^{c}	0.91^{c}	.20
8	1.47^{b}	1.44^{bc}	1.28^{c}	1.49^{b}	1.46^{b}	
16	1.95^{a}	1.91^{ab}	1.86^{b}	1.74^{b}	1.74^{b}	
24	2.04^{a}	2.12^{a}	2.06^{b}	1.79^{b}	2.35^{a}	
32	2.13^{a}	2.23^{a}	2.76^{a}	1.94^{b}	2.06^{ab}	
40	2.46^{a}	2.28^{a}	2.78^{a}	2.56^{a}	2.45^{a}	

[abc] Means within a column with unlike superscripts are significantly different ($P < .05$)
Means are averages of 2 replications
‡SEM = 0.28 (due to missing data)

Table 8. The effect of the addition of *P. acidipropionici* DH42 at different dose levels on the production of isovaleric acid during simulated in-vitro acidosis.

	Treatment Dose, cfu					
Isovaleric acid, m*M*						
Time (h)	0 (control)	10^9	10^8	10^7	10^6	SEM
0	$1.48^{c‡}$	2.44^{c}	2.34^{c}	2.28^{c}	2.06^{c}	1.30
8	4.53^{b}	4.40^{b}	4.43^{b}	3.99^{b}	3.83^{b}	
16	5.58^{ab}	5.57^{a}	5.61^{ab}	4.84^{ab}	4.71^{ab}	
24	5.60^{ab}	5.85^{a}	5.97^{a}	5.08^{ab}	5.25^{ab}	
32	6.07^{a}	5.91^{a}	6.21^{a}	5.26^{a}	5.24^{a}	
40	6.00^{a}	6.10^{a}	6.45^{a}	5.73^{a}	5.53^{a}	

[abc] Means within a column with unlike superscripts are significantly different ($P < .05$)
Means are averages of 2 replications
‡SEM = 1.38 (due to missing data)

in Kung and Hession's (1995) study are comparable to the fermentors treated with 10^9 and 10^8 cfu DH42 in the current study. Isovaleric acid exhibited a significant increase ($P<.05$) at 8 h in the control and treated fermentors. Past 8 h, no significant changes ($P>.05$) were observed as production of this remained slightly unchanged for the remainder of the 40 h fermentation period. Kung and Hession's (1995) suggestion as to the increases in branched-chain fatty acids was due to amino acid catabolism by the microorganism used in their studies. In any event, branched-chain fatty acids are essential to support the growth of many rumen bacteria (Yokoyama and Johnson, 1988) and are produced primarily by the non- cellulolytic species. This could explain the type of bacterial population present during the in-vitro fermentations in the current experiment.

The addition of strain DH42 to our fermentors resulted in no significant treatment effects ($P > .05$) on the production of total volatile fatty acids. However, within treatments, significant increases ($P < .05$) were observed at 40, 24, and 32 h in fermentors treated with 10^8, 10^7, and 10^6 cfu, respectively. The control fermentor showed a significant increase ($P < .05$) at 8 h and remained unchanged throughout the remainder of the experiment. The fermentor treated with 10^9 cfu DH42 exhibited increases in the production of total volatile fatty acids at 8 h. However, this increase was not significant ($P > .05$). The high concentrations of total volatile fatty acids observed in all fermentors could be due to the fact that the in vitro fermentation system used to conduct the current experiment was a batch system or a closed system. Therefore, an accumulation of volatile fatty acids was occurring. This is very different to what occurs in the rumen

Table 9. The effect of the addition of *P. acidipropionici* DH42 at different dose levels on the production of total volatile fatty acids acid during simulated in-vitro acidosis.

	Treatment Dose, cfu					
Total VFA, m*M*						
Time (h)	0 (control)	10^9	10^8	10^7	10^6	SEM
0	62.24[b‡]	104.98[a]	102.40[b]	102.06[b]	96.08[b]	14.18
8	142.52[a]	140.86[a]	136.25[ab]	130.31[ab]	126.61[ab]	
16	146.08[a]	143.39[a]	143.18[ab]	135.69[ab]	134.34[ab]	
24	135.44[a]	142.66[a]	142.25[ab]	133.45[a]	136.99[ab]	
32	135.74[a]	140.84[a]	142.53[ab]	138.31[a]	140.27[a]	
40	143.54[a]	143.05[a]	145.15[a]	140.07[a]	136.24[ab]	

[ab] Means within a column with unlike superscripts are significantly different ($P < .05$)
Means are averages of 2 replications
‡SEM = 20.05 (due to missing data)

where decreases in total volatile fatty acids are observed due to absorption through the rumen epithelium at low pH (Annison and Armstrong, 1970).

Preliminary studies on the ability to selectively grow strain DH42 when added to rumen fluid by using Purple Base Broth with 1% erythritol and 1.5% agar, suggested a possibility for its use as a selective and differential media (Appendix A). Therefore, this media was used for microbial analysis during the in-vitro fermentation studies with *P. acidipropionici* DH42. Microbiological analysis (Table 10) on Purple Base Broth with 1.0% erythritol and 1.5 % agar revealed growth only at 0 h (sample taken immediately after addition of wheat and dose of *P. acidipropionici* DH42) with mean recoverable numbers in the following treatments as follows: Trt 1 (10^9 cfu) 5.59 log cfu/mL; Trt 2 (10^8 cfu) 6.89 log cfu/mL; Trt 3 (10^7cfu) 7.04 log cfu/mL; Trt 4 (10^6cfu) 0.00 log cfu/mL. The table below includes the actual inoculation rate (cfu/mL) for each treatment. It is evident from the fermentor treated with 10^9 cfu (or 4.8×10^6 cfu/mL) that strain DH42 decreased in numbers as only 5.59 log cfu/mL were recovered. However, the fermentors treated with 10^8 cfu and 10^7 cfu (or 4.8×10^5 cfu/mL and 4.8×10^4 cfu/mL), DH42

numbers increased considerably. The fermentor treated with 10^6 cfu (or 4.8×10^3 cfu/mL) exhibited no growth. The increases in the numbers of strain DH42 recovered (6.89 log cfu/mL and 7.04 log cfu/mL), suggest active growth. It would have been expected to observe higher recoverable counts at the highest treatment (10^9 cfu) as this dose of strain DH42 is equal to the number of lactate utilizers normally found in the rumen. However, this discrepancy could also suggest a flaw in the methodology used to sample for strain DH42 numbers. Eventhough Trt 1 (10^9cfu) contained *P. acidipropionici* DH42 at levels equal to the naturally occurring lactate-utilizers found in the rumen during the feeding of high concentrate diets (Mackie and Gilchrist, 1979), it seems a factor was impairing its growth as it was not able to survive past 8 h in rumen fluid. Fermentor pH should not have been an environmental condition affecting the growth of strain DH42 as the minimum pH requirement for growth of propionibacteria is 5.0 (Hettinga and Reinbold. 1972). It is interesting however, to note that recoverability at 0 h in some of the fermentors coincided with the slight increases observed in propionic acid from 0 to 8 h relative to the control. This could indicate an attempt by strain DH42

Table 10. The recoverability of *P. acidipropionici* DH42 at 0 h from in-vitro fermentations with rumen fluid[h]

Treatment[k] (cfu)	Concentration/Fermentor[j] (cfu/mL)	Recovered DH42 (Log cfu/mL)	SEM
Control	(no DH42)	0.00[b]	0.833
10^9	4.8×10^6	5.59[a]	
10^8	4.8×10^5	6.89[a]	
10^7	4.8×10^4	7.04[a]	
10^8	4.8×10^3	0.00[b]	

[ab] Means with unlike superscripts are significantly different ($P < .05$)
Colony counts are averages of duplicated plates
Means are averages of two studies
[h] No recoverable numbers from 8 to 40 h
[kj] Calculated by dividing total DH42 counts (cfu) by volume of fermentor media (500 mL)

to initiate metabolic activity. No growth of the microorganism was observed past the initial sampling suggesting *P. acidipropionici* DH42 was unable to survive.

In-vivo Studies

Intraruminal administration of 40 g/kg BW ground wheat slurry as determined by preliminary animal dosing studies (Appendix B), was sufficient to produce a decrease ($P < .05$) in pH (Table 11) from 0 to 6 h within the control animals (7.02 to 5.18) and from 0 to 6 h within the treated animals (6.49 to 5.51). The amount of wheat provided to each animal via rumen fistula is shown in Appendix B. This amount is more than 10 times the amount added to each fermentor during the in vitro experiments (10 g/500 mL in vitro; approximately 107 g/500mL in vivo). The mean reduction in pH produced after the administration of the ground wheat slurry in both groups falls within the range of 5.0 to 5.5 which has been primarily associated with subacute acidosis (Goad et al., 1998). An increase in pH was observed at 8 and 10 h in the control and treated animals. However,

Table 11. The effect of *P. acidipropionici* DH42 (10^{11} cfu) on rumen pH during experimentally induced acidosis in steers.

	Treatment		
Rumen Fluid, pH			
Time (h)	Control	DH42	SEM
0	7.02^{ab}	7.40^{a}	0.22
2	5.70^{d}	6.49^{bc}	
4	5.32^{d}	5.73^{d}	
6	5.18^{d}	5.51^{d}	
8	$5.42^{d‡}$	5.62^{d}	
10	$5.64^{d‡}$	6.07^{c}	

[abcd]Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
‡SEM = 0.23 (due to missing data)

the increase was only significant ($P < .05$) in the treated animals at 10 h. An increase in rumen pH could be attributed to the metabolism of lactic acid by lactate-utilizing rumen bacteria.

Clinical signs of lactic acidosis such as diarrhea, dullness, and hyperventilation (Underwood, 1992) were not observed in acidotic cattle which were alert and mobile even though two steers had their rumens emptied due to rumen pH decreasing below 5.0 (see Appendix B). The rumens of these animals were flooded with warm water and contents were siphoned-out through a 5-cm (diameter) hose. The rumens of the two steers were inoculated with rumen contents from a healthy donor steer and offered hay. The inability to observe the characteristic clinical signs were possibly due to the fact that some animals had their rumens emptied before the onset of severe systemic acidosis or that the microbial population of those that were not emptied tolerated the carbohydrate load as the cattle were on a high concentrate feed during the whole experiment. Mackie and Gilchrist (1979) found that during a stepwise adaptation from a low to a high concentrate diet, a surge in the number of amylolytic and lactate-utilizing bacteria occurred and tended to balance each other, thus resulting in lower lactic acid accumulation. Overall, a treatment effect ($P < .05$) was observed on ruminal pH. The mean values for the control and treated animals were 5.68 and 6.13, respectively.

There were no significant differences ($P > .05$) in the control animals on the production of ruminal lactic acid within time (Table 12). There was, however, a general trend for an increase ($P > .05$) in lactic acid levels from 0 to 4 h in the control group. Treated animals exhibited a significant increase ($P < .05$) in lactic acid production from 0 to 6 h. Increases in both groups corresponded to the decreases in rumen pH during the

Table 12. The effect of the addition of *P. acidipropionici* DH42 (10^{11} cfu) on ruminal lactic acid concentration during experimentally induced acidosis in steers.

	Treatment		
Lactic acid, m*M*			
Time (h)	Control	DH42	SEM
0	0.00^{ab}	0.00^{b}	18.09
2	0.55^{ab}	10.72^{ab}	
4	27.99^{ab}	24.35^{ab}	
6	10.59^{ab}	37.52^{a}	
8	2.49^{ab}	$32.47^{ab‡}$	
10	0.10^{ab}	$36.27^{a‡}$	

[ab]Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
‡SEM = 18.93 (due to missing data)

same time frames. A decrease in rumen pH and increases in lactic acid levels are associated with the proliferation of amylolytic and lactic acid producing bacteria due to the availability of carbohydrates in the rumen (Dawson and Allison, 1988). However, lactic acid levels decreased from 8 to 10 h in both groups ($P > .05$), and corresponded to the increases in ruminal pH. This suggests that some metabolism of lactic acid was occurring. Metabolism of lactic acid in ruminants is achieved by the orderly succession of acid-sensitive and acid-tolerant genera of lactate-utilizing bacteria which have the capacity to control ruminal fermentation thereby, preventing the accumulation of lactic acid (Mackie and Gilchrist, 1979). Kung and Hession (1995) found decreasing lactic acid levels in their control cultures, but only after peaking at 5 h; cultures treated with a low dose (8.7×10^5 cfu/mL) of *Megasphaera elsdenii* B159, also exhibited the same trend. However, the high dose (8.7×10^6 cfu/mL) of the microorganism was able to control the accumulations of lactic acid. Lactic acid and pH data in the current experiment do not agree with those of Wiryawan and Brooker (1995) in which *Selenomonas ruminantium*, another lactate utilizing microorganism inoculated at 5.0 x

10^{10} cfu, was able to effectively reduce lactic acid levels in acutely grain-fed sheep. In their studies, control animals exhibited a pH of 4.9 throughout the experiment as 120 m*M* of lactic acid was produced in 8 h and no lactic acid accumulation was reported in treated animals (pH above 6.2). In the current study, lower levels or no accumulation of lactic acid were expected in treated animals because *P. acidipropionici* DH42 has the ability to metabolize lactic acid to other products such as propionic, acetic, and succinic acids (Lewis and Yang, 1992). However, there was no treatment effect ($P > .05$) as treated cultures behaved as the control cultures indicating strain DH42 was not preventing the accumulation of lactic acid.

The production of propionic acid (Table 13) in the rumen showed a significant increase ($P < .05$) at 2 h in the control animals and at 4 h in the treated animals from 0 h. After 4 h, concentrations of propionic acid in both groups were maintained steady towards the end of the trial. However, there was no treatment effect ($P > .05$) on the levels of propionic acid produced. Control animals exhibited a mean concentration of 37.7 m*M* as opposed to the treated which showed a much lower mean production of propionic acid (30.7 m*M*). This could be due to the lower levels of lactic acid found in the control animals as opposed to the treated. Wiryawan and Brooker (1995) reported increases of propionic acid from 0 to 16 h in treated animals (9.2-17.0 m*M*) as opposed to their controls that showed a gradual decrease (8.8-0.7 m*M*) within the same time frame. In contrast, in-vitro fermentations by Kung and Hession (1995) showed high productions of propionic acid in control fermentors and lower production of propionic acid in treated cultures from 0 to 9 h. Strain DH42 has the ability to produce propionic acid from lactic acid (Dawson et al., 1994).

Table 13. The effect of *P. acidipropionici* DH42 (10^{11} cfu) on propionic acid production during experimentally induced acidosis in steers.

	Treatment		
Propionic acid, m*M*			
Time (h)	Control	DH42	SEM
0	11.85^{f}	10.34^{f}	4.88
2	27.70^{de}	21.80^{ef}	
4	45.86^{abc}	36.13^{bcd}	
6	47.79^{ab}	42.27^{abc}	
8	51.55^{a}	$40.39^{abcd‡}$	
10	41.63^{abc}	$31.48^{cdef‡}$	

[abcdef]Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
[‡]SEM = 5.23 (due to missing data)

However, it is possible that the levels of propionic acid found could be attributed to other propionic acid producing rumen microorganisms such as *Veillonella alcalescens, P. acnes, Selenomonas ruminantium, Anaerovibrio lipolytica,* and *M. elsdenii* (Ogimoto and Imai, 1981).

There was no treatment effect ($P > .05$) on the ruminal production of acetic acid in the control and treated animals. Values for both groups (Table 14) ranged from 27.11 to 53.43 and 24.94 to 50.11 m*M*, in the control and treated animals respectively over the 10-hour collection period. A significant increase ($P < .05$) in acetic acid levels was observed at 8 h from 0 h in the control (27.11 to 66.22 m*M*) and treated (24.94 to 61.16 m*M*) groups with a slight tendency towards an decrease ($P > .05$) throughout the end of the sampling period. In vivo studies by Wiryawan and Brooker (1995) on grain-engorged sheep revealed an increase in acetic acid from 0 to 8 hours in treated animals as opposed to control animals in which acetic acid exhibited a decrease at 8 hours. An increase in acetic acid would have been expected in treated animals if indeed strain DH42 had

Table 14. The effect of *P. acidipropionici* DH42 (10^{11} cfu) on acetic acid production during experimentally induced acidosis in steers.

	Treatment		
Acetic acid, m*M*			
Time (h)	Control	DH42	SEM
0	27.11^{fg}	24.94^{g}	6.00
2	46.58^{de}	43.25^{ef}	
4	66.92^{a}	61.82^{abcd}	
6	66.50^{a}	66.47^{ab}	
8	66.22^{ac}	$63.27^{abc‡}$	
10	53.43^{bcde}	$52.04^{cde‡}$	

^{abcdefg}Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
‡SEM = 6.31 (due to missing data)

survived in the rumen. Acetic acid is produced in addition to propionic acid during the fermentation of lactate and glucose by propionc acid bacteria (Wood, 1981).

Treating animals with *P. acidipropionici* DH42 had no effect ($P > .05$) on the mean production of butyric acid during experimentally induced acidosis. However, butyric acid levels (Table 15) increased significantly ($P < .05$) in control animals at 2 h and in the treated animals at 4 h. Studies by Wiryawan and Brooker (1995) have shown decreases in butyric acid in control animals and increases in this acid at 8 h in treated animals. The current study does not agree with Wiryawan and Brooker's (1995) study as butyric acid increased significantly ($P < .05$) at 8 h from 2 h in the control animals and treated animals showed increases ($P > .05$) at 8 h from 4 h. The presence of butyric acid in the rumen of steers suffering from subacute acidosis have been found with increasing amounts of propionic acid (Table 13) and decreasing amounts of acetic acid (Table 14) (Goad and Nagaraja, 1988). Increasing levels of butyrate might be associated with *M. elsdenii* or *Butyrivibrio fribrisolvens*, two ruminal butyrate producers.

Table 15. The effect of *P. acidipropionici* DH42 (10^{11}cfu) on butyric acid production during experimentally induced acidosis in steers.

	Treatment		
Butyric acid, m*M*			
Time (h)	Control	DH42	SEM
0	4.52^{e}	4.65^{e}	2.01
2	7.69^{cd}	7.64^{de}	
4	10.36^{bcd}	10.30^{bcd}	
6	14.19^{ab}	12.02^{abc}	
8	15.20^{a}	$12.43^{abc‡}$	
10	12.56^{ab}	$9.19^{bcd‡}$	

[abcde] Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
[‡]SEM = 2.12 (due to missing data)

Butyrivibrio fibrisolvens has been shown to produce butyrate as a primary fermentation end product and predominates in the rumen of sheep during adaptation to a high grain diet (Mackie and Gilchrist, 1979). *M. elsdenii,* lactate-utilizer, is a major producer of butyrate from lactate metabolism in the rumen (Goad et al., 1998). Butyric acid is an important volatile fatty acid in ruminants because it synthesizes adipose and mammary gland tissue (Fahey and Berger, 1988).

There was no treatment effect ($P > .05$) on the production of succinic acid (Table 16). Within time, control animals exhibited no time interaction. However, treated animals showed a significant increase ($P < .05$) at 4 h from 0 h. At 10 h, a significant decrease ($P < .05$) in succinic acid was noted from 4 h. Succinate is the main end-product of many rumen bacteria (Yokoyama and Johnson, 1988). Lactate-utilizers such as *Selenomonas ruminantium, Veillonela alcalescens, Anaerovibrio lipolytica* and *Propionibacteria* convert succinate into propionate. It is possible that the low levels of

Table 16. The effect of *P. acidipropionici* DH42 (10^{11} cfu) on succinic acid production during experimentally induced acidosis in steers.

	Treatment		
Succinic acid, m*M*			
Time (h)	Control	DH42	SEM
0	0.12^{bc}	0.08^{c}	0.65
2	0.33^{bc}	0.54^{bc}	
4	1.07^{abc}	2.49^{a}	
6	0.82^{abc}	2.28^{a}	
8	0.38^{bc}	$1.90^{\ddagger ab}$	
10	0.19^{bc}	$0.86^{\ddagger bc}$	

[abc] Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
[‡]SEM = 0.71 (due to missing data)

succinate observed in the current study reflect an active metabolism of this end-product by lactate-utilizing species into propionate as evidenced by the increasing levels of propionic acid (Table 13) in both control and treated animals.

The treatment effect was not significant ($P < .05$) for the amounts of total volatile fatty acids produced in both the control and treated animals. Total volatile fatty acids increased significantly ($P < .05$) at 8 hours in the control group and at 6 hours in the treated group. In Wiryawan and Booker's (1995) studies, control animals showed decreases in total volatile fatty acid while the treated group showed increases. When pH is low, volatile fatty acids are absorbed from the rumen as the rumen epithelium is more permeable to the undissociated form of the acids (Stevens, 1970). This explains the decrease found in the total volatile fatty acids of control animals in the above mentioned study as rumen pH levels were low (4.9). In the current study, rumen pH was never below 5.0, suggesting that there was less absorption or slow absorption of volatile fatty acids across the rumen wall.

Table 17. The effect of *P. acidipropionici* DH42 (10^{11} cfu) on total volatile fatty acid production during experimentally induced acidosis in steers.

	Treatment		
Total VFA, m*M*			
Time (h)	Control	DH42	SEM
0	43.62^{e}	40.02^{e}	9.74
2	82.31^{d}	73.24^{d}	
4	124.22^{ab}	110.75^{ab}	
6	129.33^{ab}	123.06^{ab}	
8	133.37^{a}	$117.60^{ab‡}$	
10	107.83^{bc}	$93.19^{c‡}$	

[abcdefg] Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
‡SEM = 10.46 (due to missing data)

Blood pH (Table 18) decreased significantly ($P < .05$) at 4 h from 2 h to 7.38 from 7.43 in the control animals and was maintained steady towards the end of the experiment. Treated animals showed a significant increase ($P > .05$) in blood pH levels at 2 h. However, a significant decrease ($P < .05$) was observed at 4 h from 2 h and as in the control animals, was maintained throughout the remainder of the experimental period. A treatment effect was observed as the mean blood pH in the DH42 treated group was higher than the controls (7.42 and 7.39 m*M*). From the blood pH levels associated with this experiment, it would appear that animals were not suffering from an acute acidosis. According to Howard (1981), a blood pH less than 7.2 is an indicator of a poor prognosis along with a rumen pH of 4.5. The results in the current study are in agreement with work done by Goad et al. (1998) who reported changes in systemic acid-base status to be minimal during subacute acidosis. Their studies reported a blood pH of 7.418 at 0 h and slightly higher levels at 12-h (7.415) than those reported in the control and treated animals in the current study.

Table 18. The effect of *P. acidipropionici* DH42 (10^{11} cfu) on blood pH during experimentally inducted acidosis in steers.

	Treatment		
Blood pH Time (h)	Control	DH42	SEM
0	7.41^{bcde}	7.43^{bc}	0.016
2	7.44^{b}	7.50^{a}	
4	7.38^{de}	$7.40^{bcde‡}$	
6	7.40^{bcde}	7.40^{bcde}	
8	7.39^{cde}	7.40^{bcde}	
10	7.38^{e}	7.39^{cde}	

^{abcde}Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
‡SEM = 0.018 (due to missing data)

Treatment with DH42 had no significant ($P > .05$) effect on the levels of total blood lactate (Table 19) produced in both the control and treated animals as means were 2.16 and 2.28, respectively. Over time, no significant differences ($P > .05$) in the production of total blood lactate were observed. Total blood lactate levels are comparable to those of Wiryawan and Brooker (1998) (Table 22). They reported similarities in the total blood lactate levels for both control and treated animals. The data reported for the control animals in the current study fall within the range of total blood lactate levels (1.29 to 4.26 m*M*) reported in studies by Patra et al. (1996) (Table 22) at 0 and 12 h. In contrast, Goad et al. (1998) reported lower amounts of total blood lactate at 0 and 12 h (0.7 to 0.8 m*M*) in their studies with subacute acidosis in grain-adapted steers. Animals in the current study were adjusted to a high-carbohydrate diet. It is possible that the ground wheat offered after the 24 hour fast was sufficient to increase the amounts of total lactate present in the blood much more than the method utilized in the Goad et al.

Table 19. The effect of the addition of *P. acidipropionici* DH42 (10^{11} cfu) on total blood lactate during experimentally induced acidosis in steers.

	Treatment		
Blood lactate, m*M*			
Time (h)	Control	DH42	SEM
0	$2.04^{ab‡}$	2.17^{ab}	0.22
2	2.17^{ab}	2.19^{ab}	
4	2.29^{ab}	2.18^{ab}	
6	2.44^{ab}	2.67^{a}	
8	2.06^{ab}	2.32^{ab}	
10	1.94^{b}	2.16^{ab}	

[ab] Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
[‡]SEM = 0.25 (due to missing data)

(1998) study. Wheat is generally considered to be one of the worst grains to provoke the development of ruminal acidosis (Elam, 1976). Dunlop and Hammond (1968) have published guidelines for normal ruminant total blood lactate concentrations ranging from 0.5 to 2.0 mmol/L and Hyldgaard-Jensen and Simesen (1966; [as cited by Moller et al., 1997]) have found peak total lactic acid levels in the blood of acutely acidotic dairy cattle at 25 mmol/L. It is evident by the results obtained in the current study that neither control nor treated animals were suffering from acute acidosis. This leads to suggestion that total blood lactate levels were not high enough to overwhelm the acid-base system of the blood and the animals were therefore not systemically acidotic. Blood L(+) lactate (Table 20) of control animals in the present study were slightly higher at 0 h (5.43 mg/dL) and lower at 6 h (4.96 mg/dL) than values reported in studies by Nagaraja et al. (1985) (Table 22). Treated animals showed elevated blood L(+) lactate levels at 0 hours (6.67 mg/dL) and a significant decrease ($P < .05$) by 6 hours (4.30 mg/dL). In general,

Table 20. The effect of the addition of *P. acidipropionici* DH42 (10^{11} cfu) on blood L (+) lactate during experimentally induced acidosis in steers .

	Treatment		
L(+) lactate, mg/dL			
Time (hours)	Control	DH42	SEM
0	$5.43^{ab‡}$	$6.63^{a‡}$	0.65
2	4.19^{b}	4.39^{b}	
4	5.09^{ab}	5.30^{ab}	
6	4.96^{ab}	4.30^{b}	
8	3.98^{b}	5.78^{ab}	
10	4.10^{b}	4.26^{ab}	

[ab]Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
‡SEM = 0.72 (due to missing data)

Table 21. The effect of the addition of *P. acidipropionici* DH42 (10^{11} cfu) on blood D(-)Lactate during experimentally induced acidosis in steers.

	Treatment		
D(-) lactate, mg/dL			
Time (h)	Control	DH42	SEM
0	$13.04^{ab‡}$	$12.27^{b‡}$	2.24
2	15.40^{ab}	15.36^{ab}	
4	15.59^{ab}	14.29^{ab}	
6	17.02^{ab}	19.75^{a}	
8	14.59^{ab}	15.11^{ab}	
10	13.43^{ab}	14.24^{ab}	

[ab]Means lacking a common superscript are significantly different ($P < .05$)
Means are averages of 3 replications
‡SEM = 2.48 (due to missing data)

levels of L(+) lactate were similar for both groups and no treatment effect ($P > .05$) was observed between the control and treated animals.

D(-) lactate (Table 21) values are much higher than data published by Nagaraja et al., (1985) (Table 22). Elevated D(-) lactate values in the current study could suggest discrepancies in the method chosen to quantify this isomer. Blood D(-) lactic acid concentrations have been shown to surpass those of L(+) lactic acid but only when ruminal pH decreased below 4.5 in studies by Dougherty (1975). Nevertheless, treating the animals with strain DH42 produced no significant effect ($P > .05$) on the levels of blood D(-) lactate produced. However, there was a significant increase ($P < .05$) at 6 h to 19.75 mg/dL from 0 h in the treated group.

Table 22. The effect of the addition of 10^{11}cfu *P. acidipropionici* DH42 on total blood lactate (m*M*), L+ lactate (mg/dL), D- lactate (mg/dL), and blood pH of control and treated animals in the current acidosis study as compared to results of published data in control animals experiencing acidosis.

Time (h)	Lactate			Blood pH		References
	Current study		Published data[n]	Current study	Published data	
	Total					
	Control	Treated				
0	2.04	2.17	2.30	7.41	*	Wiryawan and Brooker, 1995
			1.29	7.41	7.42	Patra and Swarup, 1996
			0.90	7.41	7.40	Burrin and Britton, 1986
4	2.29	2.18	1.60	7.38	7.36	Burrin and Britton, 1986
8	2.06	2.32	1.70	7.39	*	Wiryawan and Brooker, 1995
			1.90	7.39	7.38	Burrin and Britton, 1986
	L(+) lactate					
0	5.43	6.63	4.40	7.41	7.41	Nagaraja et al., 1985
6	4.96	4.30	6.60	7.39	7.36	Nagaraja et al., 1985
	D(-) lactate					
0	13.04	12.27	0.10	7.41	7.41	Nagaraja et al., 1985
6	17.02	19.75	0.00	7.39	7.36	Nagaraja et al., 1985

*No blood pH reported

[n]Published data of values reported in control animals

A trial effect ($P < .05$) was observed on the average dry matter intake (DMI) as animals during trial A ate considerably more than animals in trial B (19.64 lb/d versus 17.42 lb/d). The decrease in the feed intake during trial B could suggest that the exposure to the bout of subacute acidosis in trial A could have impacted feed intake. It is known that reduce feed intake is a direct consequence of subacute acidosis (Huntington, 1988). However, treating our animals with strain DH42 produced no significant effects ($P > .05$) on the average DMI as control and treated animals ate an average of 18.33 lb/d and 18.73 lb/d, respectively. Cerna et al. (1990) have reported a slightly lower feed intake of calves when fed a microbial preparation which included a propionibacterium. The concentration of the mixture including the propionibacterium was 2×10^8 cfu/g. In a study done with swine, the feeding of propionibacterium caused a 7.2 to 46.1 % decrease in the fodder demand (Mantere-Alhonen, 1982 [as cited by Mantere-Alhonen, 1995]). Robinson et al. (1992) intraruminally inoculated fistulated steers with *Megasphaera elsdenii*, a ruminal microorganism, and found that on an average, feed intake was increased in the treated group. The concentration used was 10^{12} cfu. The current study reports no significant

Table 23. The effect of the addition of *P. acidipropionici* DH42 (10^{11} cfu) on the average dry matter intake (DMI) of steers during in vivo trials.

	Treatment		
DMI, lb/d Period	Control	DH42	Average
A	19.46	19.84	19.65[a]
B	17.21	17.62	17.42[b]
Average	18.34	18.73	

[ab] Means are significantly different ($P < .05$)
Means are averages of 3 replications
SEM = 0.7

treatment effect ($P > .05$) as the treated animals ate only 2.18% more than the control.

Microbial analysis of rumen contents revealed no presence of *P. acidipropionici* DH42 during any of the time periods (0-10 hours) in control or treated animals. A selective and differential media was used which included erythritol as a fermentation substrate, bromoscresol purple as an acidic pH indicator dye and 1.5 % agar for plating. *Propionibacterium* spp are unique in that they ferment erythritol to propionic and acetic acids (Holdeman et al., 1977). Therefore, it was thought that this unique ability would aide in the recovery and quantification of *P. acidipropionici* DH42 from rumen contents. Dawson et al. (1994) was able to estimate numbers of propionic acid-producing bacteria during studies with *P. acidipropionici* DH42 by using purple base broth with 1 % erythritol. However, their method was used as a most probable number dilution scheme. Efforts in our lab to test this medium on in-vitro and in vivo fermentations have been met with mixed results. In-vitro numbers of recoverable *P. acidipropionici* DH42 were usually higher than those recovered from steers on a high concentrate diet but not subjected to experimental acidosis (Roman, 1999 personal communication). Inhibition of growth in the ruminal environment due to acidic conditions should be excluded because ruminal pH never decreased below 5.1 during both trials. According to Hettinga and Reinbold (1972), a pH value lower than 5.0 has been proved inhibitory for the development and growth of propionibacteria. The possibility of a bacteriophage(s) or bacteriocin(s) inhibiting the growth of *P. acidipropionici* DH42 in the rumen should not be excluded. It is possible that the rumen might possess microorganisms capable of producing phages or bacteriocins that would inhibit closely related species. Bacteriophages infecting dairy propionibacteria have been isolated from Swiss-type

cheeses (Gautier, 1999) and bacteriocins produced from two species of propionibacteria have shown to inhibit *P. acidipropionici* strains (Grinstead and Barefoot, 1992; Lyon and Glatz, 1993). The inability to recover *P. acidipropionici* DH42 from the ruminal contents of acidotic steers could also be attributed to the fact that this microorganism is not a normal inhabitant of the indigenous microflora of the ruminant as it was previously isolated from silage. Fuller (1978) proposed that an effective colonization of the gut would be more readily achieved if the organism being used as a bio-inoculant (probiotic) originated from the gut itself. *P. acidipropionici* DH42 would have to effectively compete for an ecological niche in the rumen for its survival. The predominating rumen bacteria and indigenous lactate-utilizers might place the strain DH42 at a disadvantage as its would be obliged to compete for two of the more important substrates (e.g. glucose and lactate). Glucose and lactate preferences by strains of *P. acidipropionici* have been studied by several researchers (Perez-Chaia et al., 1994; Lee et al., 1974). However, the fact that no *P. acidipropionici* DH42 colonies were present on culture plates could be due to the inability of this microorganism to reproduce itself in the complex ruminal environment. Perez-Chaia et al. (1994) showed that *P. acidipropionici* exhibited low population densities and low growth rates in mixed cultures than in pure cultures. A final suggestion as to the inability of *P. acidipropionici* DH42 to survive in the rumen may be due to the fact that this microorganism was administered as a conventional freeze-dried preparation. Merry et al. (1995) have compared freeze-dried preparations of *Lactobacillus plantarum* and fresh cultures of the same strain. Although their measurements were not based on growth, they found that herbage treated with freshly cultured inoculants shortened the lag times in terms of the decline in pH than in freeze-

dried treated herbages. Freeze-dried cells are usually rehydrated prior to use and could require longer activation times after application (Merry et al., 1995). Therefore, considering that *P. acidipropionici* DH42 was introduced into a foreign environment and in a form that possibly lengthened its time to become metabolically active, the chances for its survival could have been hampered by these factors.

The results suggest that even though cattle were exposed to subacute acidosis as evidenced by a ruminal pH of no less than 5.0, *P. acidipropionici* DH42 was not able to survive in the complex microbial population of the rumen. The production of volatile fatty acids and lactic acid were probably a normal trend in the fermentation characteristics of the indigenous ruminal microflora observed in cattle experiencing subacute acidosis. Nagaraja et al. (1985) and Goad et al. (1998) both suggested that reductions in pH could be associated with higher levels of volatile fatty acids than lactic acid specifically when steers had a ruminal pH between 5.0 and 5.5.

CONCLUSION

Inoculating in-vitro rumen fermentations with different doses of *Propionibacterium acidipropionici* DH42 had a slight effect on the pH, lactic acid, and volatile fatty acids reported, but only at 0 hours. Microbiological analysis revealed the inability of strain DH42 to survive in ruminal contents as only viable colonies were detected at the first sampling and none thereafter. Thus it can only be speculated that *P. acidipropionici* DH42 produces no growth or shifts in the metabolic activities associated with rumen fluid and that a factor or factors were responsible for these observations reported.

Extrapolating the results of in-vitro studies to an in-vivo system may not adequately reproduce the same conditions found in the rumen such as ruminal volume, passage rate, and the actual microbial community present. Nevertheless, Owens and Goetsch (1988) have suggested that in-vitro findings must always be tested in-vivo as what normally is not effective in-vitro is effective in vivo. Hence, in order to test the effectiveness of our in-vitro results, *P. acidipropionici* DH42 was inoculated into the rumen at an even higher rate than in the in-vitro studies. Results showed that the treatment had no effect in producing significant changes in the fermentation profiles of lactic and volatile fatty acids, and in maintaining rumen pH near neutral between the control and treated animals. The administration of the ground wheat slurry was enough to produce subacute acidosis as evidenced by a decrease in ruminal pH to no less than 5.0. Absolutely no growth was present at any of the sampling times corresponding to the microbiological analysis during the in-vivo trials. Therefore, this leads to the suggestion that the factor or factors responsible for the inability of *P. acidipropionici* DH42 to

that the factor or factors responsible for the inability of *P. acidipropionici* DH42 to survive in-vitro were also evident when tested in a live and naturally functioning ruminal environment.

There may be various suggestions as to what may be affecting the growth and survival of *P. acidipropionici* DH42. The possibility of attack by bacteriocins could merit some consideration as it has been know that some strains of *P. acidipropionici* produce these and harm closely related species. Another consideration as to the inability of strain DH42 to survive could be due to engulfment by protozoa as they are known to engulf non-rumen bacteria. Protozoa ingest ruminal bacteria as a source of protein as well as compete for the same substrates utilized by the indigenous microflora, thus keeping bacterial numbers in the rumen in check. It is possible however, that competition of the microorganism in a batch fermentation system was hindered by the fact that *P. acidipropionici* DH42 was added as a freeze-dried commercial preparation and by the fact that propionibacteria are slow growers. Merry et al. (1995) have found that fresh cultures of *Lactobacillus plantarum* were more effective in creating shorter lag time in relation to the decline in pH needed for the stability of silage than the conventional freeze-dried treated ones. According to Merry et al. (1995), because freeze-dried microbial preparations need to be rehydrated prior to use, they probably require a longer activation time after application to herbages. It would not be incorrect to speculate that our choice of freeze-dried *P. acidipropionici* DH42 suffered these same conditions; and along with the additive effect of being slow growers, found it impossible to compete with the already established rumen microbial ecosystem.

Inoculating the rumen with lactate-utilizing microorganisms is not a novel idea and many investigators have encountered positive results. However, it should be pointed out that the microorganism utilized in those experiments are normal inhabitants of the rumen. Thus, it would be expected for these bacteria to posses a more competitive edge in their survival than a newly introduced species, as million of years of evolution have secured their ecological niche within the rumen.

Further research or improvement on the existing methods utilized in the current experiments warrant examination:

1. The use of the Tilly and Terry (1963) method for in-vitro batch fermentations as it more closely accurately represents the natural aspects of ruminal fermentation than the current method utilized.

2. Inoculating the rumen and in-vitro fermentations with fresh cultures of *P. acidipropionic* DH42 and compare results to inoculations with freeze-dried commercial preparations of the same microorganism.

3. Probe the rumen for the possibility of bacteriophages or bacteriocins exerting specific negative effects towards *Propionibacterium acidipropionici* DH42.

4. Evaluate the effects of culturing *Propionibacterium acidipropionici* DH42 with rumen protozoa and comparing the results to the survival rates of known ruminal microorganisms cultured with protozoa in-vitro.

APPENDICES

APPENDIX A

Data Used For In-vitro Studies

Table A-1. Data used for the analysis of volatile fatty acid production during in-vitro fermentation studies with *P. acidipropionici* DH42.

Reps	Time	trt	prop	lactic	acetic	butyric	isobut	valeric	isovaleric	Total VFA	pH
A	0	cont	13.12	0.00	43.22	6.38	0.86	1.16	2.52	67.24	6.63
A	0	trt1	14.80	0.00	48.50	7.73	1.06	1.34	2.83	76.26	6.64
A	0	trt2	13.65	0.00	45.45	7.02	0.94	1.24	2.59	70.89	6.64
A	0	trt3	14.55	0.00	48.04	7.47	1.02	1.33	2.82	75.22	6.62
A	0	trt4	13.17	0.00	42.03	6.37	0.90	1.11	2.47	66.06	6.67
A	8	cont	21.81	0.00	78.57	13.96	1.60	2.29	5.71	123.94	5.66
A	8	trt1	21.34	0.00	78.16	13.90	1.51	2.20	5.48	122.59	5.56
A	8	trt2	20.42	0.00	78.14	12.71	1.03	2.24	5.63	120.16	5.65
A	8	trt3	20.95	0.00	78.65	13.15	1.47	2.22	5.64	122.08	5.58
A	8	trt4	20.71	0.00	78.00	12.85	1.48	2.27	5.54	120.84	5.56
A	16	cont	25.64	0.00	89.50	17.57	1.97	2.75	6.68	144.13	5.57
A	16	trt1	25.07	0.00	87.40	17.87	1.88	2.63	6.65	141.50	5.52
A	16	trt2	24.52	0.00	90.89	16.79	1.90	2.71	6.96	143.76	5.52
A	16	trt3	23.81	0.00	86.64	15.99	1.77	2.53	6.52	137.26	5.51
A	16	trt4	24.45	0.00	90.49	16.35	20.16	2.68	6.63	142.51	5.48
A	24	cont	23.51	0.00	81.91	16.45	1.88	2.62	6.32	132.69	5.61
A	24	trt1	25.51	0.00	89.29	18.34	1.94	2.72	6.73	144.54	5.53
A	24	trt2	24.72	0.00	91.10	17.35	2.01	2.73	7.09	145.00	5.54
A	24	trt3	23.83	0.00	86.49	16.50	1.86	2.60	6.76	138.03	5.53
A	24	trt4	24.78	0.00	89.85	16.98	1.99	2.76	6.79	143.15	5.50
A	32	cont	26.55	0.00	92.45	18.88	2.20	3.00	7.27	150.34	5.57
A	32	trt1	27.58	0.00	94.98	20.10	2.23	2.99	7.27	155.15	5.51
A	32	trt2	25.35	0.00	92.88	18.29	2.08	2.82	7.19	148.62	5.51
A	32	trt3	25.51	0.00	91.81	17.90	2.05	2.92	7.07	147.26	5.51
A	32	trt4	25.98	0.00	92.41	18.60	2.19	2.96	7.21	149.34	5.48
A	40	cont	26.18	0.00	92.08	18.61	2.25	2.91	7.10	149.12	5.60
A	40	trt1	26.77	0.00	92.64	19.74	2.16	2.92	7.13	151.37	5.54
A	40	trt2	26.79	0.00	96.59	19.45	2.41	3.03	7.73	156.00	5.55
A	40	trt3	26.30	0.00	95.56	18.78	2.21	2.94	7.45	153.25	5.53
A	40	trt4	25.83	0.00	92.90	18.86	2.16	3.05	7.30	150.11	5.50
B	0	cont	.	.	.	.	.	.	.	.	5.34
B	0	trt1	36.04	0.00	71.99	20.26	1.03	2.33	2.06	133.70	5.34
B	0	trt2	35.85	0.00	72.21	20.38	1.05	2.31	2.10	133.91	5.37
B	0	trt3	36.46	0.00	67.42	20.13	0.92	2.20	1.74	128.89	5.20
B	0	trt4	36.12	0.00	65.78	19.47	0.93	2.12	1.66	126.10	5.18
B	8	cont	41.75	0.00	84.95	26.21	1.34	3.48	3.36	161.10	5.45
B	8	trt1	40.96	0.00	83.86	26.08	1.37	3.52	3.32	159.12	5.47
B	8	trt2	38.58	0.00	80.35	25.37	1.53	3.29	3.23	152.34	5.35
B	8	trt3	39.48	0.00	69.50	22.70	1.51	3.01	2.35	138.54	5.14
B	8	trt4	37.99	0.00	66.03	22.03	1.45	2.75	2.12	132.37	5.12
B	16	cont	37.56	0.03	75.87	23.67	1.94	4.52	4.48	148.02	5.45

Table A-1 (cont'd).

Reps	Time	trt	prop	lactic	acetic	butyric	isobut	valeric	Isovaleric	Total VFA	pH
B	16	trt1	36.60	0.02	74.37	23.33	1.95	4.55	4.49	145.28	5.47
B	16	trt2	35.32	0.00	73.42	23.60	1.83	4.17	4.26	142.60	5.45
B	16	trt3	38.16	0.00	65.81	21.34	1.71	3.95	3.16	134.12	5.20
B	16	trt4	36.28	0.00	61.51	20.48	1.58	3.53	2.79	126.17	5.16
B	24	cont	34.59	0.00	69.93	21.65	2.20	4.94	4.88	138.19	5.48
B	24	trt1	34.96	0.00	71.11	22.31	2.30	5.11	4.98	140.78	5.50
B	24	trt2	34.20	0.00	70.93	22.73	2.11	4.68	4.85	139.50	5.49
B	24	trt3	36.54	0.00	63.18	19.71	1.73	4.29	3.41	128.86	5.23
B	24	trt4	37.81	0.00	61.70	20.73	2.71	4.18	3.71	130.84	5.19
B	32	cont	30.30	0.00	61.18	18.50	2.07	4.21	4.87	121.13	5.51
B	32	trt1	31.38	0.00	63.92	19.67	2.24	4.75	4.56	126.53	5.50
B	32	trt2	34.02	0.00	67.07	22.02	3.45	4.63	5.24	136.43	5.50
B	32	trt3	36.46	0.00	63.19	19.53	1.83	4.90	3.45	129.36	5.26
B	32	trt4	38.04	0.00	63.19	19.90	1.94	4.87	3.27	131.21	5.21
B	40	cont	35.02	0.03	69.09	21.15	2.67	5.12	4.91	137.96	5.52
B	40	trt1	33.92	0.00	67.29	20.88	2.40	5.16	5.08	134.73	5.53
B	40	trt2	33.23	0.00	66.25	21.76	3.16	4.74	5.17	134.30	5.52
B	40	trt3	36.38	0.00	59.78	19.15	2.92	4.65	4.01	126.89	5.27
B	40	trt4	35.42	0.00	56.94	18.87	2.74	4.62	3.77	122.36	5.26

trt = treatment; cont = no DH42; trt 1 = 10^9cfu; trt 2 = 10^8 cfu; trt 3 = 10^7 cfu; trt 4 = 10^6 cfu

Table A-2. Data used for the analysis of recoverable numbers of *P. acidipropionici* DH42 during in-vitro fermentation studies.

rep	Time	trt	log cfu/ml
A	0	cont	0.00
A	0	trt1	4.04
A	0	trt2	6.18
A	0	trt3	6.30
A	0	trt4	0.00
A	8	cont	0.00
A	8	trt1	0.00
A	8	trt2	0.00
A	8	trt3	0.00
A	8	trt4	0.00
A	16	cont	0.00
A	16	trt1	0.00
A	16	trt2	0.00
A	16	trt3	0.00
A	16	trt4	0.00
A	24	cont	0.00
A	24	trt1	0.00
A	24	trt2	0.00
A	24	trt3	0.00
A	24	trt4	0.00
A	32	cont	0.00
A	32	trt1	0.00
A	32	trt2	0.00
A	32	trt3	0.00
A	32	trt4	0.00
A	40	cont	0.00
A	40	trt1	0.00
A	40	trt2	0.00
A	40	trt3	0.00
A	40	trt4	0.00
B	0	cont	0.00
B	0	trt1	7.15
B	0	trt2	7.60
B	0	trt3	7.78
B	0	trt4	0.00
B	8	cont	0.00
B	8	trt1	0.00
B	8	trt2	0.00
B	8	trt3	0.00
B	8	trt4	0.00
B	16	cont	0.00

Table A-2 (cont'd).

Rep	time	trt	log cfu/ml
B	16	trt1	0.00
B	16	trt2	0.00
B	16	trt3	0.00
B	16	trt4	0.00
B	24	cont	0.00
B	24	trt1	0.00
B	24	trt2	0.00
B	24	trt3	0.00
B	24	trt4	0.00
B	32	cont	0.00
B	32	trt1	0.00
B	32	trt2	0.00
B	32	trt3	0.00
B	32	trt4	0.00
B	40	cont	0.00
B	40	trt1	0.00
B	40	trt2	0.00
B	40	trt3	0.00
B	40	trt4	0.00

Trt = treatment; cont = no DH42; trt 1 = 10^9 cfu/ml DH42; trt 2 = 10^8 cfu/ml DH42; Trt 3 = 10^7 cfu/ml DH42; trt 4 10^6 cfu/ml DH42

Table A-3. A preliminary attempt at the recoverability of *P. acidipropionici* DH42 from in-vitro anaerobic fermentors using rumen fluid from a beef steer when plated on PBB with 1% erythritol and 1.5% agar.

	Treatments[k]		
	log cfu/ml		
Time (h)	Control	RF* and DH42	DH42 (pure)
0	0.00	0.00	8.98
6	0.00	8.51	9.18
12	0.00	0.00	9.15
18	0.00	0.00	9.35
24	0.00	0.00	9.43

[k]treatments consisted of one gram of freeze-dried DH42
*rumen fluid

Table A-4. A preliminary attempt at the recoverability of *P. acidipropionici* DH42 from in-vitro anaerobic fermentors using rumen fluid from a dairy cow when plated on PBB with 1% erythritol and 1.5% agar.

	Treatments[k]		
	log cfu/ml		
Time (h)	Control	RF* and DH42	DH42 (pure)
0	0.00	0.00	9.23
8	0.00	0.00	9.19
16	0.00	8.74	9.13
24	0.00	0.00	8.84

[k]treatments consisted of one gram of freeze-dried DH42
*rumen fluid

APPENDIX B

Data Used for In-vivo Studies

Table B-1. Data used for the analysis of fermentation end-products and pH for in-vivo studies using *P.acidipropionici* DH42 for the prevention of experimentally induced acidosis in beef steers.

Trial	trt	animal	time	lactic	prop	acetic	butyric	succinic	Total VFA	pH
A	treated	1	0	0.00	15.40	42.40	9.15	0.22	67.16	7.23
A	treated	2	0	0.00	11.60	25.39	4.33	0.18	41.50	7.15
A	treated	3	0	0.00	8.82	22.10	2.66	0.05	33.64	7.67
A	control	4	0	0.00	7.65	21.29	2.85	0.22	32.02	6.91
A	control	5	0	0.00	20.82	41.41	3.74	0.07	66.04	6.89
A	control	6	0	0.00	5.12	13.81	2.57	0.00	21.50	7.55
A	treated	1	2	0.48	26.37	67.60	15.54	0.00	109.51	6.44
A	treated	2	2	0.08	28.84	53.57	8.15	0.00	90.56	6.11
A	treated	3	2	0.00	22.89	36.70	4.50	0.09	64.17	6.74
A	control	4	2	1.48	35.68	56.83	6.80	1.12	100.43	4.90
A	control	5	2	0.00	43.93	65.93	7.22	0.00	117.09	5.91
A	control	6	2	1.87	16.57	30.21	6.40	0.07	53.25	6.83
A	treated	1	4	10.38	33.53	79.05	23.15	0.00	135.72	5.77
A	treated	2	4	5.94	39.51	63.83	10.07	0.01	113.41	5.41
A	treated	3	4	0.00	51.35	61.80	7.24	0.12	120.50	5.85
A	control	4	4	44.41	44.07	62.24	9.82	0.26	116.39	4.38
A	control	5	4	7.65	64.19	87.80	12.19	0.00	164.18	5.34
A	control	6	4	0.57	32.21	52.30	11.58	0.31	96.39	6.22
A	treated	1	6	44.77	33.66	81.12	25.73	0.00	140.51	5.72
A	treated	2	6	3.45	41.49	62.72	11.52	0.00	115.74	5.43
A	treated	3	6	0.86	70.42	77.67	10.03	0.32	158.44	5.51
A	control	4	6	50.56	35.11	52.01	9.81	0.00	96.93	4.19
A	control	5	6	2.97	70.12	91.85	15.09	0.05	177.10	5.28
A	control	6	6	0.83	44.34	66.71	16.56	0.41	128.02	5.89
A	treated	1	8	114.99	26.22	75.06	16.53	1.10	118.92	5.71
A	treated	2	8	0.00	42.87	62.07	12.38	0.00	117.32	5.60
A	treated	3	8	0.00	63.50	69.26	8.65	0.00	141.41	5.61
A	control	4	8	12.95	41.43	53.84	12.63	0.03	107.92	.
A	control	5	8	1.18	77.63	92.94	18.62	0.00	189.18	5.34
A	control	6	8	0.28	46.76	65.91	16.36	0.93	129.96	5.96
A	treated	1	10	147.55	22.84	75.30	9.46	3.96	111.56	6.24
A	treated	2	10	0.00	26.50	44.33	9.01	0.00	79.84	6.21
A	treated	3	10	0.00	47.36	52.10	6.46	0.01	105.93	6.07
A	control	4	10	0.00	29.72	42.36	10.39	0.10	82.57	.
A	control	5	10	0.00	56.90	69.36	14.19	0.03	140.48	5.54
A	control	6	10	0.28	46.76	65.86	16.36	0.92	129.90	6.69

Table B-1 (cont'd).

Trial	trt	animal	time	lactic	prop	acetic	butyric	succinic	Total VFA	pH
B	treated	1	0	0.00	15.34	30.75	5.99	0.00	52.09	7.38
B	treated	2	0	0.00	5.99	15.28	3.62	0.01	24.90	7.25
B	treated	3	0	0.00	4.94	13.72	2.17	0.04	20.87	7.73
B	control	4	0	0.00	11.16	22.61	4.92	0.16	38.84	6.80
B	control	5	0	0.00	12.67	26.15	5.30	0.23	44.34	7.17
B	control	6	0	0.00	13.72	37.43	7.78	0.08	59.01	6.80
B	treated	1	2	60.47	23.40	43.75	8.38	0.30	75.83	6.06
B	treated	2	2	3.29	14.48	27.86	5.09	2.86	59.29	6.80
B	treated	3	2	0.00	14.86	30.02	4.23	0.00	49.11	6.79
B	control	4	2	0.00	30.88	45.47	7.54	0.15	84.03	5.88
B	control	5	2	0.00	12.67	26.15	5.30	0.23	44.34	5.28
B	control	6	2	0.00	26.48	54.94	12.90	0.42	94.74	5.43
B	treated	1	4	113.33	35.47	69.94	7.68	3.25	116.34	5.39
B	treated	2	4	16.04	26.96	46.88	7.33	8.69	89.85	5.72
B	treated	3	4	0.46	30.00	49.43	6.34	2.89	88.67	6.28
B	control	4	4	1.28	47.66	60.00	8.64	2.61	118.90	5.38
B	control	5	4	0.73	51.59	69.25	12.27	0.00	133.12	5.73
B	control	6	4	113.33	35.47	69.94	7.68	3.25	116.34	4.90
B	treated	1	6	150.91	34.97	69.89	5.02	4.48	114.37	5.59
B	treated	2	6	21.68	32.74	55.33	9.89	7.70	105.66	5.23
B	treated	3	6	3.47	40.38	52.14	9.93	1.19	103.64	5.58
B	control	4	6	5.61	46.64	55.88	8.77	4.50	115.79	5.07
B	control	5	6	3.61	51.51	64.08	13.27	0.01	128.88	5.74
B	control	6	6	0.00	39.07	68.52	21.67	0.00	129.25	4.92
B	treated	1	8	.	.	.	.	.	.	5.94
B	treated	2	8	11.14	34.41	56.42	11.53	6.92	109.28	5.31
B	treated	3	8	0.06	41.94	50.64	11.28	0.25	104.10	5.55
B	control	4	8	0.57	53.81	58.02	10.27	1.27	123.36	4.80
B	control	5	8	0.00	49.29	60.92	11.86	0.00	122.06	5.82
B	control	6	8	0.00	40.41	65.74	21.51	0.06	127.72	4.96
B	treated	1	10	.	.	.	.	.	.	6.52
B	treated	2	10	0.01	39.24	53.26	11.26	0.06	103.82	5.66
B	treated	3	10	0.00	26.51	32.73	7.72	0.13	67.09	5.75
B	control	4	10	0.32	38.78	44.05	8.22	0.07	91.13	4.45
B	control	5	10	0.00	38.62	43.90	8.18	0.04	90.74	6.03
B	control	6	10	0.00	39.03	55.05	18.07	0.00	112.14	5.07

Table B-2. Data used for the analysis of total plasma lactate, L(+) lactate, and D(-) lactate concentrations and blood pH for in-vivo studies using *P. acidipropionici* DH42 for the prevention of experimentally induced acidosis in beef steers.

trial	trt	animal	time	blood pH	total blood lactate[a]	plasma L(+) lactate[b]	plasma D(-) lactate[c]
A	treated	1	0	7.43	1.73	4.44	11.16
A	treated	2	0	7.44	2.05	3.26	15.15
A	treated	3	0	7.44	2.42	7.70	14.08
A	control	4	0	7.35	2.37	3.26	18.10
A	control	5	0	7.46	1.39	5.87	6.67
A	control	6	0	7.45	.	.	.
A	treated	1	2	7.57	2.43	3.39	18.45
A	treated	2	2	7.62	2.18	4.05	15.57
A	treated	3	2	7.50	2.33	2.87	18.07
A	control	4	2	7.46	1.49	3.26	10.12
A	control	5	2	7.47	2.15	3.65	15.73
A	control	6	2	7.40	2.61	3.78	19.68
A	treated	1	4	7.38	2.09	4.70	14.08
A	treated	2	4	7.36	1.91	4.18	12.98
A	treated	3	4	7.39	2.35	3.00	18.12
A	control	4	4	7.28	2.52	6.53	16.16
A	control	5	4	7.40	2.37	4.05	17.31
A	control	6	4	7.40	2.39	2.74	18.80
A	treated	1	6	7.39	2.03	4.05	14.25
A	treated	2	6	7.37	2.13	4.18	15.02
A	treated	3	6	7.39	4.61	3.13	38.33
A	control	4	6	7.26	2.31	6.66	14.16
A	control	5	6	7.40	2.85	3.92	21.71
A	control	6	6	7.39	2.36	3.78	17.46
A	treated	1	8	7.42	2.57	5.48	17.68
A	treated	2	8	7.41	2.81	3.92	21.41
A	treated	3	8	7.40	1.72	9.66	5.82
A	control	4	8	7.28	2.56	3.52	19.52
A	control	5	8	7.43	1.67	2.87	12.19
A	control	6	8	7.39	1.59	3.65	10.69
A	treated	1	10	7.39	1.17	4.70	5.86
A	treated	2	10	7.38	2.55	3.26	19.72
A	treated	3	10	7.39	2.14	8.22	11.04
A	control	4	10	7.26	2.42	4.70	17.08
A	control	5	10	7.40	1.97	3.00	14.70
A	control	6	10	7.40	2.00	3.00	15.00
B	treated	1	0	7.45	2.58	7.18	13.10
B	treated	2	0	7.44	1.96	9.27	8.37
B	treated	3	0	7.43	.	.	.

Table B-2 (cont'd).

trial	trt	animal	time	blood pH	total blood lactate[a]	plasma L(+) lactate[b]	plasma D(-) lactate[c]
B	control	4	0	7.40	2.58	4.31	18.91
B	control	5	0	7.41	1.81	4.83	11.43
B	control	6	0	7.44	2.20	10.05	9.75
B	treated	1	2	7.45	1.99	4.44	13.44
B	treated	2	2	7.44	1.65	4.44	10.38
B	treated	3	2	7.45	2.61	7.18	16.28
B	control	4	2	7.43	2.33	4.70	16.24
B	control	5	2	7.41	2.02	5.87	12.31
B	control	6	2	7.44	2.47	3.92	18.35
B	treated	1	4	.	2.02	7.18	11.00
B	treated	2	4	7.43	2.75	5.61	19.11
B	treated	3	4	7.45	1.96	7.18	10.46
B	control	4	4	7.44	2.14	6.53	12.74
B	control	5	4	7.41	1.90	6.00	11.10
B	control	6	4	7.37	2.46	4.70	17.44
B	treated	1	6	7.41	2.51	8.09	14.53
B	treated	2	6	7.44	2.81	1.57	23.75
B	treated	3	6	7.45	1.94	4.83	12.63
B	control	4	6	7.44	2.64	4.31	19.45
B	control	5	6	7.44	2.39	5.74	15.74
B	control	6	6	7.44	2.11	5.35	13.61
B	treated	1	8	7.41	2.38	5.22	16.20
B	treated	2	8	7.42	2.69	5.22	19.02
B	treated	3	8	7.39	1.75	5.22	10.56
B	control	4	8	7.40	2.76	4.70	20.14
B	control	5	8	7.42	3.03	4.96	22.34
B	control	6	8	7.42	0.76	4.18	2.66
B	treated	1	10	7.39	3.01	5.74	21.38
B	treated	2	10	7.42	2.07	4.70	13.90
B	treated	3	10	7.41	2.06	4.96	13.58
B	control	4	10	7.41	1.35	6.13	6.05
B	control	5	10	7.41	2.32	4.05	16.83
B	control	6	10	7.39	1.63	3.78	10.92

[a]concentration expressed as m*M*; analyzed with HPLC

[b]concentration expressed as mg/dl; analyzed with Sigma Kit 826-B

[c]concentration expressed as mg/dl; difference between total and L(+) lactic acid

Table B-3. The amount of wheat necessary to provoke ruminal acidosis and reduce ruminal pH below 5.0 when added as a wheat slurry (1 part wheat:2 parts water) via rumen cannula to 4 steers during preliminary studies.

	Treatments			
	pH			
Time (hours)	10 g/kg	20 g/kg	30 g/kg	40 g/kg
0	6.65	6.39	7.31	7.18
4	5.89	5.3	6.68	5.62
6	5.88	5.22	4.92	5.13
9	**	4.73	*	*
10	6.83	*	*	*

*data available or not available because of evacuation of rumen contents
**missing data

Table B-4. The amount of wheat used to induce experimental acidosis during In-vivo trials when added as a slurry (1 part wheat:2 parts water) and prepared at 40 g/kg body weight of steers.

Animal	Body Weight*	Wheat
	(kg)	(kg)
A1	440.0	5.86
A2	442.0	5.89
A3	500.0	6.66
A4	483.0	6.44
A5	471.0	6.28
A6	533.0	7.11

*weights of animals represent the average of 2 trials.

Table B-5. Data used for the analysis of Dry Matter Intake (DMI) for steers during trial A and trial B of experimental acidosis.

Trial	Animal	Offered	W'back	Intake	DMI	Dry matter
A	#331	35.80	0.00	35.80	26.30	73.45
		42.26	7.10	35.16	25.83	
		39.04	6.00	33.04	24.27	
		35.80	7.70	28.10	20.64	
		32.60	0.90	31.70	23.28	
		39.04	5.20	33.84	24.86	
		39.04	8.10	30.94	22.73	
		35.80	9.60	26.20	19.24	
		32.60	0.00	32.60	23.94	
		32.60	7.80	24.80	18.22	
		30.40	0.00	30.40	22.33	
		35.80	5.40	30.40	22.33	
				Avg	22.83	
	#007	32.60	0.00	32.60	23.94	
		39.04	9.70	29.34	21.55	
		35.80	7.00	28.80	21.15	
		32.60	8.50	24.10	17.70	
		30.40	4.60	25.80	18.95	
		30.40	0.00	30.40	22.33	
		35.80	2.80	33.00	24.24	
		35.80	5.90	29.90	21.96	
		35.80	11.80	24.00	17.63	
		32.60	7.80	24.80	18.22	
		30.40	0.00	30.40	22.33	
		35.80	5.40	30.40	22.33	
				Avg	21.03	
	#274	28.20	4.30	23.90	17.55	
		28.20	1.30	26.90	19.76	
		30.40	6.50	23.90	17.55	
		28.20	8.90	19.30	14.18	
		24.96	0.00	24.96	18.33	
		30.40	5.80	24.60	18.07	
		30.40	1.40	29.00	21.30	
		28.20	0.00	28.20	20.71	
		32.60	8.00	24.60	18.07	
		30.40	4.20	26.20	19.24	
		30.40	8.40	22.00	16.16	
				Avg	18.27	
	#231	24.96	6.20	18.76	13.78	
		22.74	0.00	22.74	16.70	
		28.20	4.00	24.20	17.77	
		28.20	5.10	23.10	16.97	
		28.20	6.10	22.10	16.23	
		24.96	3.70	21.26	15.62	
		24.96	3.80	21.16	15.54	
		24.96	5.10	19.86	14.59	

Table B-5 (cont'd).

Trial	Animal	Offered	W'back	Intake	DMI	Dry matter
		24.96	5.10	19.86	14.59	73.45
		24.96	2.06	22.90	16.82	
		22.74	1.90	20.84	15.31	
		24.96	9.00	15.96	11.72	
				Avg	15.55	
	#214	42.26	0.00	42.26	31.04	
		47.70	14.30	33.40	24.53	
		42.26	18.10	24.16	17.75	
		35.80	10.60	25.20	18.51	
		32.60	4.00	28.60	21.01	
		32.60	8.00	24.60	18.07	
		30.40	6.00	24.40	17.92	
		28.20	0.00	28.20	20.71	
		32.60	21.30	11.30	8.30	
		30.40	0.00	30.40	22.33	
		35.80	5.50	30.30	22.26	
				Avg	20.22	
B	#331	42.26	14.50	27.76	20.03	72.15
		39.04	14.80	24.24	17.49	
		35.80	5.10	30.70	22.15	
		32.60	7.70	24.90	17.97	
		30.40	0.60	29.80	21.50	
		35.80	3.70	32.10	23.16	
		35.80	8.20	27.60	19.91	
		32.60	4.20	28.40	20.49	
		32.60	2.10	30.50	22.01	
		32.60	0.20	32.40	23.38	
				Avg	20.81	
	#007	35.80	12.10	23.70	17.10	
		32.60	15.20	17.40	12.55	
		30.40	1.20	29.20	21.07	
		32.60	10.40	22.20	16.02	
		30.40	2.30	28.10	20.27	
		30.40	0.00	30.40	21.93	
		32.60	2.40	30.20	21.79	
		32.60	3.60	29.00	20.92	
		32.60	3.50	29.10	21.00	
		32.60	5.50	27.10	19.55	
				Avg	19.22	
	#274	30.40	10.40	20.00	14.43	
		28.20	13.10	15.10	10.89	
		24.96	0.60	24.36	17.58	
		30.40	10.90	19.50	14.07	
		28.20	8.70	19.50	14.07	
		24.96	3.20	21.76	15.70	

Table B-5 (cont'd).

Trial	Animal	Offered	W'back	Intake	DMI	Dry matter
		24.96	5.90	19.06	13.75	72.15
		22.74	2.20	20.54	14.82	
		22.74	4.90	17.84	12.87	
		22.74	0.80	21.94	15.83	
				Avg	14.40	
	#231	24.96	1.10	23.86	17.21	
		28.20	12.00	16.20	11.69	
		24.96	5.20	19.76	14.26	
		22.74	5.20	17.54	12.66	
		19.52	0.80	18.72	13.51	
		24.96	2.00	22.96	16.57	
		24.96	6.10	18.86	13.61	
		22.74	6.00	16.74	12.08	
		19.52	1.90	17.62	12.71	
		22.74	2.40	20.34	14.68	
				Avg	13.90	
	#214	35.80	7.10	28.70	20.71	
		32.60	11.20	21.40	15.44	
		30.40	1.20	29.20	21.07	
		32.60	5.20	27.40	19.77	
		30.40	7.20	23.20	16.74	
		28.20	2.10	26.10	18.83	
		28.20	8.40	19.80	14.29	
		24.96	0.90	24.06	17.36	
		30.40	5.60	24.80	17.89	
		28.20	3.00	25.20	18.18	
				Avg	18.03	

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