THE EFFECTS OF DOSING FEEDLOT CATTLE WITH *MEGASPHAERA ELSDENII* STRAIN NCIMB 41125 PRIOR TO THE INTRODUCTION OF A GRAIN-RICH DIET

by

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Abstract

Two experiments were conducted to evaluate the efficacy of *Megasphaera elsdenii* strain NCIMB 41125 and its potential use in the mitigation of ruminal acidosis. In experiment 1, a metabolism study was conducted to evaluate ruminal parameters, quantify changes in ruminal bacterial populations, and determine *in vitro* capacity for lactate utilization following intraruminal dosing of a placebo or *M. elsdenii* strain NCIMB 41125 and an abrupt diet change. Angus crossbred steers (n=20; average BW=253 ± 24 kg) fitted with ruminal cannulas were blocked by BW and assigned randomly to treatments. Treatments consisted of intraruminal dosing with a placebo (100 mL of autoclaved culture), or 10, 100, or 1,000 mL of a live culture containing 1.62×10^{8} CFU/mL of *M. elsdenii* strain NCIMB 41125. Prior to inoculation, cattle were placed into individual pens in an enclosed facility and allowed free access to alfalfa hay, salt, and water. Feed and water were removed for 24 h prior to administering treatments, after which, cattle were allowed free access to a diet consisting of 34% alfalfa hay and 66% steam-flaked corn-based concentrate. On d 7, cattle were fed an 80% concentrate diet. On d 12, steers were started on the final finishing diet of 94% concentrate. Ruminal pH and concentrations of lactate and VFAs were monitored following introduction of each concentrate diet. Ruminal samples were collected at 0, 2, 4, 6, 8, and 24 h after feeding for quantitative rt-PCR detection of native and introduced strains of *M. elsdenii*, as well as total bacterial genomes. Capacity for metabolism of lactic acid was evaluated by inoculating 0.2 mL of strained ruminal fluid into anaerobic culture tubes containing 15 mL of semi-defined lactate medium. Tubes were incubated at 39˚C, and turbidity changes were determined by measuring absorbance at 2 h intervals up to

12 h. Experiment 2 was conducted in a commercial feedlot to evaluate the efficacy of *M. elsdenii* strain NCIMB 41125 for improving feedlot performance. A second objective of the study was to determine if oral dosing of *M. elsdenii* has the potential for reducing the number of cattle treated for bovine respiratory disease. Angus steers and heifers (n = 3179; average BW = 356 ± 58.4kg) were used in a randomized complete block design with two treatments. Cattle were assigned to treatment on an every-other-head basis such that every-other-animal was orally drenched with 100 mL of a culture medium containing 1.5×10^{8} cfu/mL *M. elsdenii* strain NCIMB 41125 at processing. Cattle were blocked by gender and date of arrival. To maximize profitability, cattle were sorted via visual appraisal to identify cattle that were market ready. Cattle were shipped to a commercial abattoir in Lexington, NE for harvest. Data obtained for each pen of cattle included feedlot performance, morbidity, mortality, carcass characteristics, and grid-based program carcass qualifications. In trial 1, compared to the placebo group, cattle administered *Megasphaera* maintained higher ruminal pH 24 h after the carbohydrate challenge ($P < 0.05$). Ruminal lactate concentrations increased in response to the diet change ($P < 0.05$), but concentrations were lower for cattle that received *Megasphaera* compared to the placebo group ($P < 0.05$). Total number of bacterial genomes 24 h after inoculation was unaffected by intraruminal dosing of *M. elsdenii* strain NCIMB 41125 ($P > 0.05$), but populations of undifferentiated *M. elsdenii* and strain NCIMB 41125 increased by 24 h after inoculation ($P < 0.05$). Turbidity of cultures inoculated with ruminal fluid increased in response to *M. elsdenii* administration ($P < 0.05$), suggesting a greater capacity for lactate utilization in inoculated cattle compared to the placebo group. In trial 2, no differences in feed efficiency were observed ($P > 0.05$). Compared to cattle dosed with *Megasphaera*, the control group had more USDA yield grade 2 carcasses ($P < 0.05$), and cattle dosed with *M. elsdenii* had more USDA yield grade 5

carcasses ($P < 0.05$). *Megasphaera* cattle also tended to have more USDA Prime carcasses ($P = 0.14$). No effects on incidence of liver abscesses were observed. Dosing cattle with *M. elsdenii* prior to introduction of typical concentrate diets may be useful in preventing ruminal lactate accumulation and associated depressions in ruminal pH. Inoculating cattle with *M. elsdenii* is effective in bolstering populations of ruminal lactate utilizers, and may be useful in preventing ruminal lactate accumulation in grain-fed cattle. No effects on reducing episodes of BRD were noted.

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Dedication

I would like to dedicate this thesis to my grandfather. I can only hope to be the man you are! Your love for agriculture will forever shine in all that I do. You are the cornerstone in my life and I thank God for each opportunity that you've given me.

"Bring hither the fatted calf, and kill it; and let us eat, and be merry; For this my son was dead, and is alive again; he was lost and is found." Luke 15:23-24

"He causeth the grass to grow for the cattle, and herbs for the service of man, that he may bring forth food out of the earth." Psalms 104:14

Non sibi sed allis

Preface

The work herein is a mere beginning to the path that I intend to travel. It is paved with good intentions. I know that the paths ahead of me will not always be traversed with ease. It is my fullest intention to press on in the strive for making a positive impact on society through an improved agriculture. I have found my calling and the roots that make me who I am are planted firmly in a field that will be forever a part of everything I do. I hope to give back what it has given to me. I pray that each and every humbling experience will enrich my ability to do so.

Devote yourself wholeheartedly towards the direction of the young and your returns on investment will pay off exponentially. All for the sake of supporting what I believe in and for the promotion of agriculture well into the future. You have to believe. There comes a time when hope must be transformed into something which is tangible. I just feel that one day it will be in my calling to life to gather the minds of those who think alike and take the lead into promoting in a collaborative effort the ideals of agriculture above and beyond what our predecessors established. A road that will be paved with the blood sweat and tears of those who were, who are, and who are yet to be leaders of a livelihood precious to us all.

CHAPTER 1 - Literature Review

Introduction

The cattle feeding sector of United States animal agriculture has evolved into a system that employs intensive management and the use of grains as a primary source of energy. High energy densities of grain has accommodated feedlots in such a way that they are less expensive per unit of energy compared to forages. Grains also are easier to store, process, mix, and deliver to feed bunks than forages. Unfortunately, accommodations are met with challenges. Ruminal acidosis in feedlot cattle continues to be a common digestive disorder and can lead to marked reductions in cattle performance (Nagaraja and Titgemeyer, 2007). Acidosis frequently occurs in feedlots with cattle fed high-energy diets (Britton and Stock, 1989). Since newly-arrived feedlot cattle normally are not adapted to high-grain diets, the highest propensity for a digestive upset occurs during the adaptation period to high energy finishing rations typically employed by the cattle feeding industry. During adaptation to diets high in non-structural carbohydrates, there is a shift in the ruminal microbial flora. Fibrolytic bacteria become less prominent and amylolytic species increase in number. The microorganisms inhabiting their rumens reflect populations that are suited to digestion of forages rather than non-structural carbohydrates. Combined with a change in diet composition, the ecological shift in the rumen of feedlot cattle can perturb normal rumen function. Feedlot cattle also experience a great deal of stress during marketing and upon arrival at the feedlot (Hutcheson and Cole, 1986; Loerch and Fluharty, 1999). Transportation to a feedlot can be stressful for many reasons (Grandin, 1997). The stress of food and water deprivation during procurement and transportation to the feedlot may have a substantial impact on feed intake following arrival (Brown et al., 2006). The combined effects of stress from

transportation and deprivation of feed and water also can disrupt ruminal microbial populations (Galyean et al., 1981), which may exacerbate nutritional and metabolic problems in newly arrived feedlot cattle. Adaptation of cattle previously fed forage-based diets to diets rich in concentrates is known to cause marked changes in the ruminal environment, and time is an important factor to consider when allowing the ruminal microflora to adapt to these changes (Bevans et al., 2005). The required adaptation period may take from two to four weeks, during which time digestive disturbances such as acidosis, diarrhea, and bloat may occur. These conditions often result in poor performance, morbidity, and death.

Use of Cereal Grains in Feeding Cattle and Implications for Ruminal Acidosis

Ruminant animals and ruminal microorganisms have a symbiotic relationship that facilitates fiber digestion, but domestic ruminants in developed countries are often fed an abundance of grain and little roughage (Russell and Rychlik, 2001). The economics of cattle feeding are directly related to animal performance, and use of grains in feedlots is very prominent. High levels of animal productivity cannot be sustained by forage alone (Nocek, 1988).

Maximizing Energy Intake

In the cattle feeding business, emphasis has been placed on maximizing energy intake above maintenance to produce the most efficient gains (Stock et al., 1990). In today's marketplace, the cost per megacalorie of net energy for maintenance (**NE_m**) or net energy for gain (**NE_g**) of dietary ingredients favors feeding high-concentrate diets based on cereal grains or grain byproducts (Brown et al., 2006). Typical feedlot diets of the United States consist of total mixed rations (**TMR**) containing 50 to 95% grain, and thus are rich in readily fermentable carbohydrates (**RFC**). The use of grain-rich rations has increased productivity of finishing cattle,

allowing producers to become more profitable in a business that is inherently risky due to very narrow margins of profitability.

Grain Processing Methods

The implementation of various grain processing methods has augmented the increased productivity associated with grain feeding. The primary goal of processing is to increase energy (starch) availability (Owens et al., 1997). Starch in grains can be made more readily available by processing methods which are commonly used by feedlots. Unfortunately, the improvements in productivity do not come without cost. The potential for acidosis is most likely the greatest single factor that limits advances in grain processing (Owens et al., 1997), because further processing often increases digestibility and rate of ruminal fermentation. Feeding cattle high-grain diets has brought concurrent problems with ruminal acidosis, and grain overload in feedlot cattle has gained the most attention because of its economic impact (Castillo et al., 2004).

Extent of the Acidosis Problem and its Economic Impact

Acidosis in feedlot cattle is among the problems most frequently associated with feeding high-grain rations. Acidosis has been well known ever since grain feeding became a widespread practice (Nagaraja and Lechtenberg, 2007). In fact, digestive problems are the second-most common ailment among feedlot cattle in the United States known to cause morbidity and mortality (Agriculture Statistics Board, National Agricultural Statistics Service, U.S. Department of Agriculture, 2006). According to the National Agricultural Statistics Survey (**NASS**) of 2006, digestive problems resulted in the loss of 648,000 head of cattle, or 16.8% of all deaths, with an economic impact of about $367.4 million. Actual economic losses associated with acidosis are difficult to assess (Stock, 2000) due to limitations in our ability to diagnose subacute manifestations of the disorder. Diet change is one of the most significant environmental stressors

for cattle shipped to feedlots. Proper diet management is of particular importance when receiving cattle and introducing grain into their diet.

Defining Acidosis

Modern cattle feeders view acidosis as a significant nutritional disorder that is a consequence of feeding diets that contain large proportions of rapidly-fermented carbohydrates with low roughage content. Ruminal acidosis, or increased accumulation of organic acids in the rumen, reflects imbalances between microbial production, microbial utilization, and ruminal absorption of organic acids (Nagaraja and Titgemeyer, 2007). Ruminal acidosis is due to the excess ingestion of feeds rich in RFC. Such feeds contain large amounts of starch, sucrose, lactose, or glucose (Hungate et al., 1952). Acidosis has been defined as the biochemical and physiological stresses caused by rapid production and absorption of ruminal organic acids and endotoxins caused by the overconsumption of readily fermentable carbohydrates (Britton and Stock, 1987). Britton and Stock (1987) also added that acidosis is not one disease, but a continuum of degrees of ruminal disorders. Acidosis in a feedlot results when cattle consume fermentable carbohydrates in amounts sufficient to cause a nonphysiologic accumulation of organic acids in the rumen, with a concurrent reduction in pH (Nagaraja and Titgemeyer, 2007). Acidosis has been associated with many factors that are known to have a major impact on the feedlot industry. Feeding cattle high grain diets that are conducive to development of ruminal acidosis has been charged as a causative factor in founder, polioencephalomalacia, and rumenitis (Brent, 1976). Acidosis also impacts the mechanics of rumen function, causing stasis and rumenitis, both of which have deleterious effects on rumen motility (Glock and DeGroot, 1998). Others have linked ruminal acidosis to sudden death syndrome, reduced feed intake, reduced nutrient absorption, liver abscesses, grain bloat, and clostridial infections (Britton and Stock,

1987). In beef cattle fed high-concentrate diets, the ability of the animal to buffer the rumen is minimal due to limited mastication and rumination, resulting in insufficient salivary secretion (Carter and Grovum, 1990).

In order to make description less complicated, acidosis has been divided into two categories based on the presence or absence of overt clinical signs (Nagaraja et al., 1998). Britton and Stock (1987) characterized the degrees of acidosis as acute and subacute. Subacute and acute acidosis have different characteristics. Among these are biochemical, physiological, and microbiological changes within the rumens of afflicted animals.

Subacute Acidosis

Changes within the rumen are common in subacute acidosis, but are not as drastic as with acute acidosis. The capacity of the ruminal bacterial population to utilize starch as a substrate remains constant, with a change in end products. The bacteria that inhabit the rumen produce volatile fatty acids, which accumulate at a rate faster than can be absorbed. Subacute acidosis has been described as subclinical acidosis and is less well defined than acute acidosis (Vasconcelos and Galyean, 2008). In 1965, Dirksen first mentioned the concept of subclinical acidosis. He characterized the condition as chronic latent ruminal acidosis, and claimed that its occurrence was more common than that of acute acidosis. Subacute ruminal acidosis can be defined as a condition where pH is between 5.0 and 5.5, concentrations of short-chained fatty acids are increased, and the ratio between acetic, propionic, and butyric acid has shifted to favor more propionate and butyrate, and where concentrations of lactic acid do not exceed 5 to 10 m*M* (Hibbard et al., 1995). Subacute ruminal acidosis is a health and production problem that is common in the cattle industry of the United States when cattle are fed diets rich in RFC to promote high rates of growth (Nordlund, 2003). Feedlot managers often associate acidosis with

only acute acidosis due to the fact that the symptoms are observable, while the major manifestation of subacute acidosis is reduced feed intake (Britton and Stock, 1989). Reduced feed intake directly affects profitability of cattle feeding since it is associated with performance.

Acute Acidosis

Changes associated with acute acidosis include effects on systemic and ruminal function, and have been reviewed extensively (Howard, 1981). Acute acidosis in ruminants is the result of consuming excess amounts of fermentable carbohydrates, which causes a non-physiological reduction in pH and the production of a toxic factor(s) (Slyter, 1976). In response to introduction of RFC into the diet, the resident microbial population begins to shift. In acute acidosis, the rumen becomes populated by lactic acid producing bacteria. Hibbard et al. (1995) defined acute acidosis as being consistent with ruminal pH less than 5.0 with accumulation of lactic acid greater than 90 m*M*. Acute ruminal acidosis has been characterized with ruminal pH levels less than 5.0 or 5.2 (Vasconcelos and Galyean, 2008). Acute ruminal acidosis may develop in animals that are unfamiliar with concentrate diets, or when they consume moderate excesses of rapidly fermented diets to which they have been adapted (Dirksen, 1965). The propensity for feedstuffs to induce lactic acidosis is dependent on the presence of lactic acid precursors (Kersting et al., 2009).

Animals suffering from acute acidosis may be sick to the point of death, or exhibit impaired physiological function such as absorption (Huntington and Britton, 1979; Bauer, 1992). Death may occur within 24 to 72 hours following grain engorgement in serious cases. In acute ruminal acidosis, blood flow to the gastrointestinal tract is reduced, thereby decreasing the absorption of all organic acids from the rumen, thus lowering ruminal pH (Stock, 2000). Non-physiologic accumulation of organic acids resulting in a low ruminal significantly impacts

microbial activity, rumen function, health, and ultimately animal productivity (Nagaraja and Lechtenberg, 2007). If such problems are prolonged or not ameliorated, the low ruminal pH can damage the rumen wall, thereby affecting absorptive capacity.

Clarifying the Differences

Years of painstaking research have given scientists, ruminant nutritionists, and producers the knowledge needed to define the differences between acute and subacute acidosis. Nagaraja and Titgemeyer (2007) summarized differences between subacute and acute acidosis in beef cattle (Table 1-1 and 1-2). Clinical signs and mortality are common occurrences that are often present in acute acidosis, where they are not in subacute cases. Major differences among the two ailments are the changes in ruminal pH, and concentrations of VFA and lactate. Ruminal flora changes in acute cases are more abrupt than in subacute cases. Populations of Gram-negative bacteria are decreased in acute acidosis, while Gram-positive species proliferate substantially. The lactate-producing bacteria *Streptococcus bovis* increases in acute ruminal acidosis, while lactate-utilizing species decrease. With a decrease in lactate-utilizing bacteria **(LUB),** lactate accumulates in the rumen during acute lactic acidosis. Table 1-1 illustrates these relationships. Since the rumen is a key site of absorption of the products of fermentation, acidosis also has effects on blood chemistry. In cattle with acute or lactic acidosis, the decline in rumen pH and increase in lactic acid ultimately impacts blood pH. Blood pH in cattle that are suffering from acute acidosis can drop below the critical level of 7.35. In response to decreased blood pH, bicarbonate is lowered due to the innate effort of the body trying to buffer the abnormal pH. Yet another change in blood characteristics is an increase in packed cell volume reflecting the flow of water from blood into the rumen. Ruminal osmotic pressure increases due to increased organic acid concentrations within the rumen (Huber, 1976), and water from the circulatory system

diffuses into the rumen, resulting in concentration of red blood cells. Table 1-2 summarizes the major differences in systemic effects of acute and subacute acidosis in feedlot cattle.

Etiology of Acidosis

The causes and origins of acidosis in ruminants have been well researched and explained (Britton and Stock, 1987; Huntington, 1988; Elanco, 1993; Harmon, 1996). We know that in high-producing feedlot cattle being fed high-grain diets, the risk of developing acidosis is high. Upon introduction of RFC into the diet, the propensity for development of acidosis is increased by changes in rumen microbial populations and ruminal fermentation, which can lead to changes within the animal. Figure 1-1 depicts the changes that can occur in feedlot cattle. Figure 1-2 diagrams the ruminal events that lead to acidosis.

The Introduction of Grain

Feedlots currently employ feeding methods to safely increase the concentrate portion of the diet in relatively short periods, but perturbations frequently result in the development of acidosis (Elam, 1976). Experimental models have been used to demonstrate that development of acidosis is associated with introduction of grain into the diet. Uhart and Carroll (1967) induced acidosis in steers with an abrupt diet change from an alfalfa hay diet to a ration containing 90% concentrate. In response to the development of acidosis, the steers went “off feed”, ruminal pH declined, and ruminal lactic acid concentrations were elevated.

Microbial Changes

Numerous changes take place within the rumen during the onset of acidosis. Extensive changes in the bacterial population of the rumen are among these circumstances. Microbial digestion in the rumen can be influenced by changing the contents of the rations. Amylolytic

species of bacteria can make up as much as 90 to 95% of total culturable bacteria in grain-fed animals (Leedle et al., 1982). Overconsumption of highly-fermentable carbohydrates is followed by a selective proliferation of ruminal micro-organisms, increasing the concentration of organic acids and decreasing ruminal pH, which can sometimes be fatal (Dirksen, 1965). *Selenomas ruminantium*, *S. bovis*, and anaerobic *lactobacilli* are ruminal bacteria that proliferate very well in grain-fed cattle, which may contribute to the rapid accumulation of DL-lactic acid and VFA (Nagaraja and Titgemeyer, 2007). The number of Gram-positive bacteria increases in grain-fed animals. Fermentative and microbial changes that occur are not as abrupt in subacute acidosis as in acute acidosis (Britton, 1984). Microbial changes in the rumen favor shifts to increased numbers of Gram-positive lactic-acid producing bacteria, destruction of the Gram-negative bacteria, and a decrease or complete defaunation of ciliated protozoa (Nagaraja et al., 1998). Protozoa are functional in providing a buffering effect in the rumen because they are predacious to bacteria, which decreases bacterial activity and slows rate of starch fermentation (Nagaraja et al., 1992). The initial phase of acidosis includes rapid growth of major lactic acid-producing bacteria such as *Streptococcus bovis* and *Lactobacillus* spp. (Hungate et al., 1952; Krogh, 1961; Mann, 1970; Allison et al., 1975; Slyter, 1976). Since lactate is an intermediate product of ruminal fermentation that can be further metabolized into VFA, the presence of LUB in cattle fed grain is common. In ruminants being fed diets rich in RFC, *Megasphaera elsdeni, Propionibacterium acnesi,* and *Selenomas ruminantium* ssp. *lactilytica* are the most common LUB (Russell and Dombrowski, 1980).

Fermentation Changes

Harmon et al. (1985) contrasted differences in ruminal absorption and concentrations of organic acids produced during experimentally induced subacute and acute acidosis. Subacute

acidosis was induced via a rapid diet change from a forage diet to one containing 70% concentrate. Acute acidosis was induced by infusing glucose directly into the rumen. In response to the treatments, rumen pH declined in both groups of animals. In the subacute treatment, ruminal pH was higher (5.8) than in the acute treatment (4.2). Concentrations of L(-) lactate rose to 80 m*M* in the acute glucose-infused cattle, while only small increases were noted in the subacute treatment. Similar patterns were observed in both treatments for D(+) lactate. Britton and Stock (1987) hypothesized that the large increase in lactate with acute acidosis treatment was a result of increased synthesis, exceeding the capacity for degradation. Lactic acid has a pKa of 3.1 (Dawson et al, 1997) and is a stronger acid than the principal ruminal VFA (pKa 4.8). Rumen stasis is a concern with ruminal pH near 5.0, and is the result of central nervous system inhibition by hydrogen ion receptors elsewhere in the gastrointestinal tract, absorbed acids, amines, and toxins (Huber, 1976). The decline in ruminal pH can be attributed to lactic acid accumulation and decreased lactic acid fermentation in animals experiencing acute acidosis, and the accumulation of VFA in subacute cases (Nagaraja and Titgemeyer, 2007).

Changes in the Animal

As a result of ruminal acidosis, cattle may experience a plethora of secondary problems including rumenitis, acidemia, toxemia, endotoxemia, and bloat (Glock, 1998). Common systemic changes that have been noted are hemoconcentration, decreased blood pH, lowered blood bicarbonate, and increased L(+) and D(-) lactate concentrations (Nagaraja et al., 1998). Decreased ruminal pH and extreme acidic conditions can lead to more complex problems in the rumen, including parakeratosis. Absorption becomes impaired, and bacteria can cross the ruminal wall and enter the bloodstream. Liver abscesses are a common result of such changes to the ruminal wall (Nagaraja and Chengappa, 1998). Other ailments have been associated with

acidosis in feedlot cattle, including founder, polioencephalomalacia, and rumenitis (Brent, 1976). Britton and Stock (1987) included sudden death syndrome, malabsorption, and clostridial infections to the list of ailments commonly occurring in cattle suffering from acidosis.

Signs and Symptoms

According to Kleen et al. (2003), subacute and acute acidosis are different degrees of the same problem. Signs and symptoms of acidosis are important for producers to be familiar with so that treatment may begin and mitigation of the cause may proceed.

Subacute Acidosis

Clinical symptoms are not evident in subacute cases of ruminal acidosis (Stock and Britton., 1993). The most notable symptom is reduced feed intake, which may not be detected when animals are fed in groups. Laminitis is a common externally observed symptom (Nocek, 1997).

Acute Acidosis

The acute stage of ruminal acidosis is more harmful and severe to the animal because physiological functions can be impaired (McSweeney and Mackie, 1997). Cattle experiencing acute acidosis are depressed, ataxic, off-feed, have dilated pupils, and tachycardia is common. Diarrhea is a common occurrence, and cattle may die within 2 to 5 days following the insult (Nordlund, 1995).

Management of Cattle to Prevent Acidosis

Preventing acidosis in feedlot cattle is a major component of sound nutritional management (Elam, 1976). The inclusion of roughages in feedlot rations may be effective in preventing acidosis. Since roughages are expensive, difficult to handle, and low in energy,

inclusion levels often are very low. Feed additives, including ionophores, direct-fed microbials (**DFM**), and buffers are commonly used by feedyards to reduce the risk of acidosis. Non-ionophore antibiotics and fat supplementation also have been suggested for prevention of acidotic insults.

Diet Adaptation

Newly arrived feeder cattle normally are not adapted to diets rich in RFC. The diet adaptation period is considered by many to be a critical time at which nutritional management strategies may either promote or impair subsequent feedlot performance and health (Brown et al., 2006). It is a widely practiced management strategy to introduce cattle to grain gradually over a few weeks, with the amount of grain in the diet increasing over time (Klieve et al., 2003). A process of adapting ruminal microorganisms to rations high in RFC is necessary because an abrupt transition is conducive to metabolic disorders (Cheng et al., 1998; Owens et al., 1998), which may result in long term deleterious consequences or death (Krehbiel et al., 1995; Nagaraja and Chengappa, 1998). One definition of an adapted ruminant is the point at which an animal can be fed a concentrate diet which would be likely to induce acidosis in an unadapted animal, with no adverse effects (Counotte and Prins, 1981). The traditional method of adapting cattle to high-grain diets includes sequential diets with increasing grain concentration and decreasing forage concentration in each step, which ensures that ruminal microorganisms gradually adjust to a ruminal environment with a lower pH (Choat et al., 2002). Other methods to help enhance adaptation have been developed such as using a consistent time of daily feed delivery (Schwartzkopf-Genswein et al., 2004), limit-feeding (Murphy et al., 1994), and greater frequency of feeding (Soto-Navarro et al., 2000). Nutritionists should consider numerous

variables and convey nutritional management strategies that will insure optimization of the cost of gain during adaptation and finishing (Brown et al., 2006).

Feed Additives

Acidosis is known to have important economical impact due to its effects on health and productivity issues. Scientists have developed methods of reducing the risks of acidosis. Methods for manipulating ruminal fermentation include ionophores, antibiotics, and direct-fed microbials (Wallace, 1994). In the mid-1970's, ionophores were approved for use in ruminants in the United States by the Food and Drug Administration (Russell and Strobel, 1989). Ionophores are lipophilic polyether antibiotics that are inhibitory to some rumen organisms (Russell and Strobel, 1989), and are known for their ability to reduce the risk of lactic acidosis (Owens et al., 1998) and bloat in feedlot cattle (Cheng et al., 1998). Nagaraja et al. (1982) found that lasalocid appeared to be most effective in preventing acidosis compared to monensin or thiopeptin. Dennis and Nagaraja (1981) reported that lasalocid sodium and monensin sodium alter rumen fermentation by inhibiting the lactate-producing bacteria in the rumen.

Antibiotics

Recently, the use of antibiotics for livestock has been thought to contribute to the emergence of microorganisms which are resistant (Yabuuchi et al., 2007). Continuous pressure by consumers and special interest groups that regard the use of antibiotic additives in livestock rations as contributing factors in antimicrobial resistance is an important issue facing producers. Definitive links have not been proven (Russell and Rychlik, 2001), but producers, nutritionists, and animal scientists should be savy in their techniques to avoid potential problems. It has been suggested that efforts attempting to procure effective natural feed additives should be made (DiLorenzo et al., 2006).

Microbial Feed Additives

Naturally occurring, live bacterial supplements are known as probiotics or direct-fed microbials (**DFM)** (Yoon and Stern, 1995). Their use offers another method of altering rumen fermentation in order to reduce the risk of ruminal acidosis is the use of DFM. Direct-fed microbials are microorganisms that are administered to cattle orally. A sundry of microbial feed additives are commercially available to livestock producers (Beharka and Nagaraja, 1998). Feeding bacterial DFM is based on the concept of providing positive post-rumen effects on the animal by improving the population of beneficial gut microflora (Beauchemin et al., 2003). Bacterial DFM have the potential to be used in combination with a complete nutritional strategy to reduce the incidence of acidosis in feedlot cattle being fed rapidly fermentable diets (Ghorbani et al., 2002). Using *in vitro* and *in vivo* studies, inoculating cattle with *Megasphaera elsdenii* has been shown to modify ruminal fermentation and prevent accumulation of lactic acid during diet changes, not unlike what is typical in newly-received feedlot cattle (Greening et al., 1991; Kung and Hession, 1995). When considering the use of *M. elsdenii* as a DFM, of paramount concern is the ability of the organism to amplify and establish a viable ruminal population (Ouwerkerk et al., 2002). Increased presence of LUB in the rumens of cattle being fed grain-based diets could prove beneficial as a result of their ability to prevent accumulation of lactate (Nisbet and Martin, 1994; Kung and Hession, 1995). Using DFM that stimulate ruminal LUB could provide an alternative method to reduce the negative economic impacts associated with feeding grain-rich diets to feedlot cattle (Waldrip and Martin, 1993). Kung and Hession (1995) conducted an *in vitro* study to determine if *Megasphaera elsdenii* would prevent the accumulation of lactic acid during the introduction of a high concentrate diet. Ruminal fluid taken from a hay-fed steer was mixed with a buffer, and rapidly degradable substrates. The experiment was conducted in triplicate with an uninoculated flask, a low dose flask of *M. elsdenii* (8.7×10^5 cfu/mL), or a high

dose flask (8.7×10^6 cfu/mL). Inoculation with *M. elsdenii* prevented an excessive drop in pH and the accumulation of lactic acid. They determined that inoculation with *M. elsdenii* has the potential to prevent lactate accumulation when cattle are fed diets high in RFC (Kung and Hession, 1995). There is very limited published information on the mechanisms by which DFM improved performance in cattle being fed high-grain diets (Ghorbani et al., 2002). Responses to DFM in ruminants often are small and variable, and much research still needs to be done to establish dosage size and application route (Kung, 2001). Work to evaluate the role of various probiotics like yeast, and specific strains of bacteria on the development of metabolic disorders such as acidosis should be continued (Galyean and Eng, 1998).

The History of *Megasphaera elsdenii*

Elsden and Lewis (1953) described a large, VFA-producing, strictly anaerobic, non-motile cocci that they isolated from a sheep. Elsden et al. (1956) isolated another similar-appearing organism approximately 2.4 × 2.6 μm in diameter, which occurred in pairs and in chains up to 20 cells in length. A similar organism was discovered in the rumens of bloating cattle by Gutierrez et al. (1959) and they proposed the name *Peptostreptococcus elsdenii*. In 1971, Rogosa proposed the transfer of *P. elsdenii* to a new genus, *Megasphaera*.

An Introduction to *Megasphaera elsdenii*

Megasphaera elsdenii is a Gram-negative, non-motile, large cocci that produces fatty acids and is strictly anaerobic (Elsden and Lewis, 1953). *M. elsdenii* ferments lactate via the acrylate pathway (Waldrip and Martin, 1993). *M. elsdenii* can ferment both L+ and D- isomers of lactate through use of the enzyme racemase, and may be a key contributor in the control or prevention of lactic acidosis by removing lactic acid (Stewart and Bryant, 1988). Based on this

theory, *M. elsdenii* could prove to be a useful probiotic when directed to decreasing the occurrences of acidosis and in improving the efficiency of starch utilization in the rumen of grain-fed cattle (Kung and Hession, 1995; Wiryawan and Brooker, 1995; Owens et al., 1998). Counotte et al. (1981) found that *M. elsdenii* was the predominant LUB within the rumen, fermenting up to 97% of ruminal lactate. Robinson et al. (1992) reported that steers dosed intraruminally with *M. elsdenii* had reduced lactate concentrations, higher ruminal pH, and 24% greater DMI when compared to control animals. In another experiment conducted by Hibbard et al. (1993), oral drenching with *M. elsdenii* prevented lactic acidosis and improved intake of cattle switched from a 50% to 90% concentrate diet. *M. elsdenii* could be a useful organism when used to inoculate feedlot cattle for the prevention of lactate accumulation in the rumen as well as shortening the time for adaptation to high-grain diets (Kung and Hession, 1995).

Where is the organism found?

M. elsdenii is naturally occurring in the rumens of cattle. It has been shown that ruminal populations of *M. elsdenii* are diet dependent, and increases are normal with the introduction of grain to the diet (Mackie and Gilchrist, 1979).

Biochemistry of *Megasphaera elsdenii*

The large cocci commonly found in the rumens of grain-fed cattle has a unique method of utilizing lactate. *M. elsdenii* can ferment both optical isomers of lactate that are produced in the rumen. Figure 1-3 diagrams the chemical structures of D (-) and L (+) lactate.

Lactate is is an intermediate of ruminal fermentation. L(+) is the predominant isomer, but D(-) usually increases as ruminal pH decreases (Giesecke and Stangassinger, 1980). *M. elsdenii* has the ability to take advantage of either form of lactate through the use of the enzyme racemase (Asanuma and Hino, 2002). The production of propionate is important to ruminant physiology

and nutrition because it is glucogenic (Hino and Kuroda, 1993). Propionate can be produced by two pathways, the acrylate and the randomizing pathway. In grain-fed ruminants, the acrylate pathway predominates and accounts for 70-90% of propionate produced (Russell and Gahr, 2000). Fermentation of carbohydrates in the rumen prevents large amounts of glucose from being absorbed from the digestive tract of ruminants (Bergman et al., 1970; Huntington and Reynolds, 1986). Gluconeogenesis is continuous and a major metabolic activity (Bergman, 1990). The only VFA making a significant net contribution to glucose is propionate, and it is the most important single precursor of glucose (Bergman et al., 1966; Bergman, 1982; Bergman, 1983). Past researchers have reported that *M. elsdenii* is the most important lactate utilizer in the rumen (Counotte et al., 1981). Because such an ability is important to feedlot producers, *M. elsdenii* could possibly be a key factor in reducing the occurrences of acidosis (Russell et al., 1981).

The Acrylate Pathway

Ruminal lactate may be removed by passage beyond the rumen, absorption from the rumen, or by microbial fermentation (Counotte et al., 1981). With respect to microbial fermentation, *M. elsdenii* is capable of fermenting both isomers of lactate to propionate and acetate by employing the acrylate (nonrandomizing) pathway (Gottschalk, 1979). The formation of propionate via the acrylate pathway frequently is associated with the rumen, and its importance in the conversion of lactate is interesting (Stevani et al., 1991). Counotte et al. (1981) concluded that *M. elsdenii* ferments up to 97% of lactate produced in the rumen. When carbohydrates are included in the diet, most DL-lactate utilizers do not ferment DL-lactate at the same time (Russell and Baldwin, 1978) because of catabolite repression. Other lactate utilizers are known to ferment lactate via the succinate pathway. It is not yet known whether or not

M elsdenii is the only ruminal microbe to possess the acrylate pathway (Prins and Stewart, 1997). Figure 1-4 diagrams the acrylate pathway.

Deamination of Amino Acids

Elsden et al. (1956) stated that *M. elsdenii* is a carbohydrate-fermenting ruminal bacteria with the ability to ferment amino acids. The organism's primary role within the rumen is the utilization of lactate (Counotte et al., 1981). From a nutritional standpoint, it appears that *M. elsdenii* may prove functional when used to bolster ruminal populations of LUB. Deamination of amino acids in the rumen is a nutritionally wasteful process (Rychlik et al., 2002). Before considering use of *M. elsdenii* as a DFM, it may be important to verify that bolstering populations of the organism in the rumen will not be conducive to producing excess amounts of ammonia. Past work revealed that *M. elsdenii* produced significant amounts of branch-chained VFA if glucose was limiting (Allison, 1978).

Use of *Megasphaera elsdenii* as a Direct Fed Microbial

Manipulation of the ruminal microbial ecosystem in a way such that production efficiency is increased has been a challenge to ruminant nutritionists and rumen microbiologists (Yoon and Stern, 1995). Safe and effective methods for control and prevention of acidosis in feedlot cattle could have valuable economic, animal health, and animal welfare implications. Since *M. elsdenii* is an important LUB, it has practical application for use as a probiotic to prevent and control ruminal acidosis (Ouwerkerk et al., 2002). *M. elsdenii* could have a substantial role in the prevention or control of acidosis by reducing ruminal lactate concentrations (Stewart, and Bryant, 1988).

Conclusion

Accumulation of ruminal lactate is an inherent risk of feeding diets rich in grain. Research has shown that the use of *Megasphaera elsdenii* as a probiotic can reduce lactate accumulation. Inoculating cattle with *M. elsdenii* could be effective in bolstering populations of LUB. Dosing cattle with *M. elsdenii* prior to the introduction of a concentrate diet may successfully prevent the accumulation of lactic acid and resulting acidosis.

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Table 1-1. Comparison of the changes in ruminal parameters, and ruminal microbial populations of beef cattle affected by acute and subacute ruminal acidosis[1,2].

	Acidosis	
Item	Acute[3]	Subacute[4]
Ruminal pH	<5.0	5.0 to 5.5
Total organic acid concentration	Increased	Increased
Lactic acid concentration	High (50 to 120 m*M*)	Normal (0 to 5 m*M*)
VFA concentration	Below normal (<100 m*M*)	High (150 to 225 m*M*)
Type of Microbe		
Gram-negative bacteria	Decreased	No change
Gram-positive bacteria	Increased	No change
Streptococcus bovis	Increased initially	No change
Lactobacillus spp.	Increased	Increased
Lactic acid-producers	Increased	Increased
Lactic acid-utilizers	Decreased	Increased
Ciliated protozoa	Absent or decreased	Absent or decreased

[1]Adapted from Nagaraja and Titgemeyer (2007).
[2]Data from Nagaraja et al. (1998).
[3]Changes in response variables are in relation to forage-adapted animal.
[4]Changes in response variables are in relation to grain-adapted animal.

Table 1-2. A comparison of the systemic changes in beef cattle affected by acute and subacute ruminal acidosis[1,2].

	Acidosis	
Blood parameter	Acute[3]	Subacute[4]
pH	Decreased (<7.35)	Normal to slightly decreased
Lactic acid	Increased, particularly D(-)	Normal
Bicarbonate	Marked reduction, (20 mEq/L)	Normal to transient reduction
Base status	Base deficit	Base excess
Packed cell volume	Increased (>40%)	Normal (30 to 35%)
Endotoxins	Yes	Yes
Inflammatory mediators	Yes	Yes

[1]Adapted from Nagaraja and Titgemeyer (2007).
[2]Data from Nagaraja et al. (1998).
[3]Changes in response variables are in relation to forage-adapted animal.
[4]Changes in response variables are in relation to grain-adapted animal.

Figure 1-1. Changes that occur with diet change and the onset of acidosis.

The figure serves as a flow diagram of events that occur when cattle are fed diets rich in readily fermented carbohydrates. Higher inclusion levels of grain may elicit similar responses. Following the introduction of grain, a microbial shift occurs in the rumen. As a result, fermentation changes take place. If acidosis develops and persists, cattle may experience systemic changes which can further impair health status.

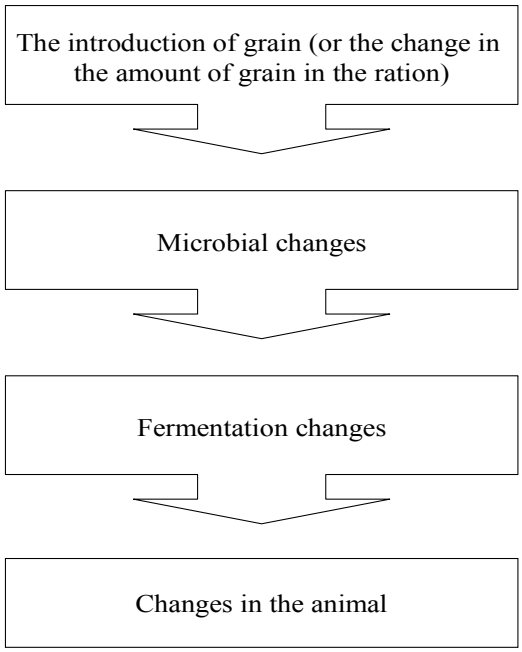
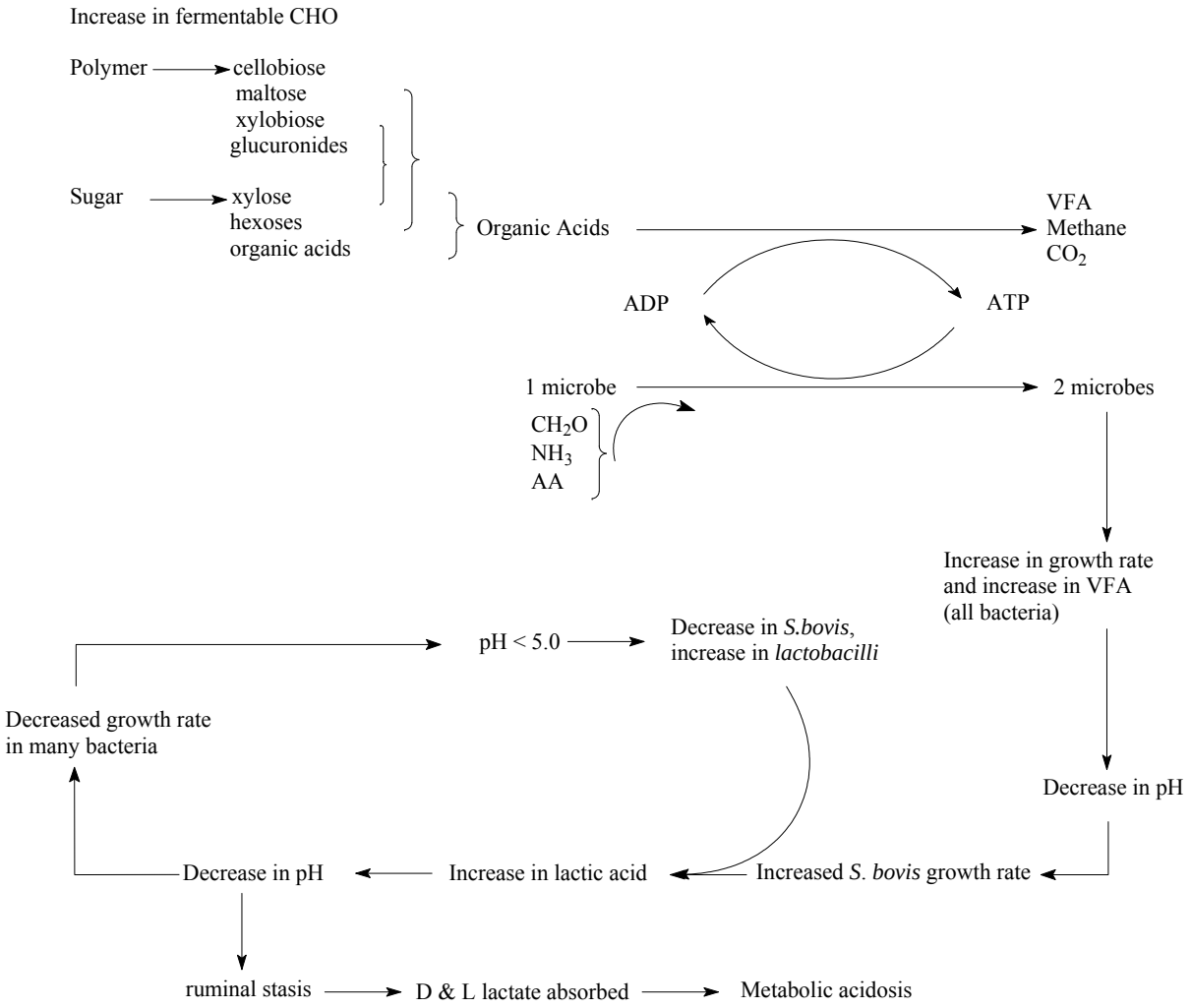
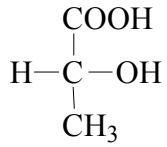


Figure 1-2. The etiology of acidosis.

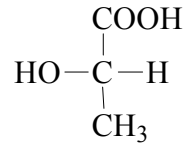


Adapted from (Baldwin and Allison, 1983; Nocek, 1997)

Figure 1-3. Optical isomers of lactate.

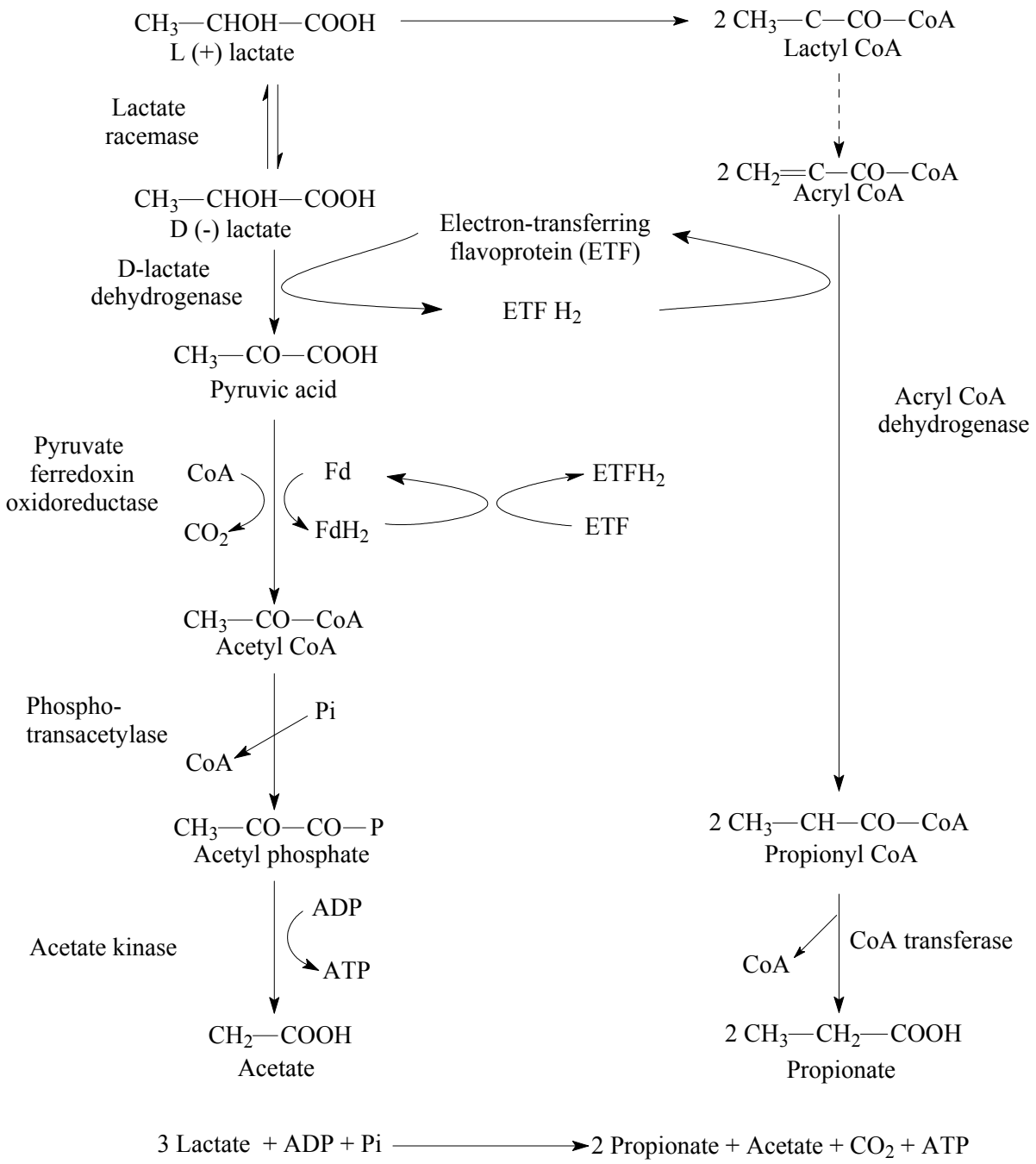


D (-) Lactic Acid



L (+) Lactic Acid

Figure 1-4. The acrylate (nonrandomizing) pathway.



Adapted from Gottschalk (1979).

CHAPTER 2 - Investigation of the effects of *Megasphaera elsdenii* strain NCIMB 41125 on ruminal pH and ruminal concentrations of organic acids following a carbohydrate challenge

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This is contribution No. _____ from the Kansas Agricultural Experiment Station, Manhattan. KS

Abstract

A metabolism study was conducted to evaluate ruminal parameters in cattle following an abrupt change to a high carbohydrate diet and oral administration of a placebo or varying doses of *Megasphaera elsdenii* strain NCIMB 41125. Angus crossbred steers (n=20; average BW=253 ± 24 kg) fitted with ruminal cannulas were blocked by BW and assigned randomly to treatments. Treatments consisted of intraruminal dosing with a placebo (100 mL of autoclaved culture), or 10, 100, or 1,000 mL of a live culture containing 1.62×10^{8} cfu/mL of *Megasphaera elsdenii* NCIMB 41125. Prior to inoculation, cattle were placed into individual pens in an enclosed facility and allowed free access to alfalfa hay, salt, and water. Feed and water were removed 24 h prior to administering treatments. On the morning of the initial diet change, cattle were administered their treatments and then allowed free access to a diet consisting of 34% alfalfa hay and 66% steam-flaked corn-based concentrate. On d 7, cattle were stepped up to an 80% concentrate diet, and on d 12, were stepped up to a 94% concentrate diet. Ruminal pH and concentrations of lactate and VFAs were monitored following introduction of each diet. Compared to the placebo group, cattle administered *M. elsdenii* maintained higher ruminal pH 24 h after the initial carbohydrate challenge. Ruminal lactate concentrations increased in response to the initial diet change ($P < 0.05$), but concentrations were lower for cattle that received *Megasphaera* compared to the placebo group ($P < 0.05$). On d 17, following a 24 h fasting period, cattle were refed and ruminal lactate concentrations remained lower among cattle in the 1000 mL treatment group ($P < 0.05$).Dosing cattle with *M. elsdenii* immediately prior to introduction of typical concentrate diets may be useful in preventing ruminal lactate accumulation and associated depressions in ruminal pH.

Introduction

Acidosis in feedlot cattle is among the most frequently occuring problems associated with feeding high-grain diets. The greatest propensity for digestive upset occurs during the period of adaptation to high energy finishing diets. During adaptation to diets high in non-structural carbohydrates, there is a shift in the ruminal microbial flora. Fibrolytic bacteria become less prominent and amylolytic species increase. Combined with a change in diet composition, the ecological shift in the rumen of feedlot cattle can lead to abnormal rumen function. The pH of rumen fluid in forage-fed cattle usually is in the range of 5.8 to 6.8, but rapid introduction of readily fermented carbohydrates can quickly lower pH to < 5.0 (Bergman, 1990). The rapid decline in ruminal pH associated with excessive consumption of non-structural carbohydrate-rich diets may overwhelm buffering and absorptive capacity, thus leading to acidosis. Increased production of organic acids by a proliferative population of amylolytic bacteria is a key contributor to the decline of ruminal pH in grain-fed cattle. Ruminal acidosis, or accumulation of organic acids in the rumen, reflects an imbalance between microbial production, microbial utilization, and ruminal absorption of organic acids (Nagaraja and Titgemeyer, 2007). Concentrations of VFA in the rumen are highly variable depending on diet and time after feeding, although the total amount present usually is between 60 and 150 m*M* (Bergman, 1990). A decrease in ruminal pH increases the absorption of undissociated VFA across the ruminal epithelium (Danielli *et al.*, 1945). The production of acids, followed by their utilization, neutralization, or absorption from the rumen is a dynamic process. Volatile fatty acids are not the only organic acids produced in the rumen. When cattle are being fed high-concentrate diets, lactate is another common fermentation end product of some ruminal bacteria such as the starch utilizer, *Streptococcus bovis* (Slyter, 1976; Waldrip and Martin, 1993). If rate of lactate

production exceeds rate of absorption or fermentation, accumulation can result in ruminal acidosis. The accumulation of lactate within the rumen can lead to reduced pH and a decrease in the efficiency at which feed is converted to VFAs and microbial protein (Ouwerkerk *et al*., 2002). Lactate is present in the rumen as both L (+) and D (-) isomers. A significant increase in bacteria capable of utilizing both isomers of lactate could ameliorate problems associated with excess concentrations of lactic acid. *Megasphaera elsdenii* is a Gram-negative, non-motile, large coccus that produces fatty acids and is strictly anaerobic (Elsden and Lewis, 1953). ***M. elsdenii*** can ferment both isomers of lactate through use of the enzyme racemase, and may be function in the control or prevention of lactic acidosis by removing lactic acid (Stewart and Bryant, 1988). *Megasphaera elsdenii* could prove to be a useful probiotic when directed towards decreasing the occurrences of acidosis and improving the efficiency of starch utilization in grain-fed cattle (Kung and Hession, 1995; Wiryanwan and Brooker, 1995; Owens et al., 1998).

Materials and Methods

All procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol no. 2535).

Cultures of ***M. elsdenii*** strain NCIMB 41125 were grown in continuous culture under anaerobic conditions at 39˚C and pH maintained at 5.5 using a Sartorius BIOSTAT® C-DCU (Sartorius BBI Systems, Melsungen, Germany) bioreactor with a dilution rate of 40%. A semi-defined lactate medium was used to support growth of the inoculum. Nitrogen gas was used to maintain anaerobic conditions. Bacterial cultures were harvested into stainless steel kegs, and administered to cattle within 24 h of harvest.

Twenty Angus crossbred steers (average BW=253 ± 24 kg) were purchased from a local sale barns for use in this study. Upon arrival at the Beef Cattle Research Center, steers were

offered *ad libitum* access to alfalfa hay and fresh water before initial processing. At initial processing BW were obtained and steers were vaccinated for viral and clostridial diseases (Bovi-shield 4 and Ultrabac 7, Pfizer Inc.; Exton, PA) and given an external parasiticide (Phoenectin; IVX Animal Health., St. Joseph, MO). Steers were fitted with ruminal cannulas (Bar Diamond, Parma, ID), and housed within an indoor facility containing individual slatted-floor pens measuring 1.5 m × 3 m each. Pens were equipped with individual feed bunks and automatic water fountains, allowing *ad libitum* access to alfalfa hay, salt, and clean water throughout a 3-wk recovery period.

Following recovery from surgery, steers were blocked by BW and assigned randomly, within blocks, to one of four treatments. Treatments consisted of oral dosing with a placebo (100 mL of autoclaved culture), or 10, 100, or 1,000 mL of a live culture containing 1.62×10^{8} cfu/mL of *Megasphaera elsdenii* NCIMB 41125.

To allow for comparable post-feeding sampling times for all animals, the first animal in the row of 20 pens was fed at 0800 h, and successive animals were fed at 5-min intervals thereafter. This allowed sufficient time for feeding, inoculating, and sampling, thus making it possible to retrieve ruminal digesta at pre-determined intervals after feeding. The same feeding and sampling routine was followed throughout the trial (Figure 2-1). On d 1, background samples were taken immediately prior to feeding. On d 2 through 7, samples of ruminal digesta were taken at 0, 2, 4, 6, 8 and 24 h post-feeding. On d 12 and 17, samples were taken at 0, 2, 4, 6 and 8 h post-feeding. On d 18, 24, 31, and 42 samples were taken only at 4 h post-feeding. At each sampling time, animals were haltered and tethered to the side panel of the stall. The ruminal fistula cap was removed, and four handfuls of ruminal contents were removed from the cranial and ventral sacs of the rumen and placed into a sample cup and subsequently strained through

four layers of cheesecloth. Immediately following straining, the pH of the sample was determined using an Orion Model 420A pH meter (Orion Co., Boston, MA) calibrated with pH 4.0 and 7.0 standards (Fisher Chemical Co., Fairlawn, NJ).

Precisely 4 mL of strained ruminal fluid were pipetted from the sample cup and placed into a scintillation vial with 1 mL of 25% (wt/vol) metaphosphoric acid solution. Vials were capped, mixed, and placed on ice. Samples were then transported to the laboratory and frozen at -20˚C. Upon thawing, the acidified ruminal fluid samples were centrifuged at 15,000 × g for 15 min. One mL of the clear supernatant was pipetted into a 12 mm × 75 mm tube to which 50 µL of 6N NaOH were added. Pivalic acid was included as an internal standard. One mL of the prepared sample was then transferred to a gas chromatography vial to be analyzed. The vials were immediately capped and frozen for subsequent analyses of lactate and VFA concentrations using a gas chromatograph (Hewlett-Packard 5890A, Palo Alto, CA) equipped with an auto-injector (200˚C injection temperature) and a packed column (2 m × 2 mm column; Carbopack B-DA 80/120; 4% CW 20 m column packing; Supelco, Bellefonte, PA) with He as the carrier gas at a flow rate of 24 mL/min, a column temperature of 175˚C, and flame ionization detector temperature of 200˚C. Total VFA production at each sampling time was calculated as the sum of the individual VFA molar concentrations. The acetate/propionate ratio was computed by dividing molar concentration of acetate by the molar concentration of propionate.

In order to establish baseline ruminal values prior to exposure of cattle to concentrates, background samples were taken at 0800 h on d 1 of the experimental period. Steers were then fasted for 24 h. Beginning at 0800 h on d 2, ruminal digesta was collected from each animal prior to feeding (0 h), and the appropriate treatment was subsequently administered as a liquid inoculum via the ruminal cannula. Treatments included 10 mL; 100 mL; and 1000 mL of fresh

culture, providing 1.62×10^{9}; 1.62×10^{10}; and 1.62×10^{11}, respectively, of live *M. elsdenii* strain NCIMB 41125. A placebo consisting of 100 mL of autoclaved culture was administered to the control group. Immediately after sampling and dosing, steers received concentrate-based feed for the first time. Steers were given *ad libitum* access to a steam-flaked corn-based diet composed of 66% concentrate and 34% ground alfalfa hay (Table 2-1). On d 7, cattle were changed to a diet consisting of 80% concentrate, again using steam-flaked corn as the principal energy source and alfalfa hay as the roughage source (Table 2-1). Beginning on d 12, steers were fed the final finishing ration consisting of 94% concentrate and 6% alfalfa hay (Table 2-1). Feed disappearance at each sampling point was recorded, thus making it possible to monitor daily DMI for each animal during the experiment.

Statistical Analysis

Data for ruminal pH, lactate and VFA concentrations, and DMI were analyzed as repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model statement included effects of treatment, sampling time, and the interaction of these effects. Weight block served as the random effect. Contrasts included a comparison of the placebo group to the average of all groups administered live *Megasphaera elsdenii* NCIMB 41125, as well as the linear and quadratic effects of dosage size.

Results and Discussion

Background Information

On d 1 of the study, ruminal pH and concentrations of VFA prior to introduction of concentrates were similar among steers assigned to the different treatment groups. During this time, steers were fed only roughage.

Animal Health

During the course of the study, animal health was monitored according to Kansas State University Beef Cattle Research Center standard operating procedures. Any animals exhibiting signs of illness were given immediate attention and treated according to standard operating procedures. On the morning of d 21, a steer from the 10 mL live culture treatment group presented symptoms of dyspnea, lethargy, and anorexia. Following medical treatment, the steer died on d 22 at 1330 h, and was promptly submitted to the veterinary diagnostic laboratory for necropsy. The steer succumbed to severe bronchopneumonia. On d 28, a steer from the 100 mL live culture treatment group exhibited dyspnea. After medical treatment, the steer died on d 30. The steer was taken to the veterinary diagnostic laboratory for necropsy where it was diagnosed with severe bronchopneumonia.

Post-Inoculation Ruminal pH and Feed Intake

Rapid introduction of a readily fermentable carbohydrate is known to decrease ruminal populations of fibrolytic bacteria and allow amplification of amylolytic bacteria (Goad *et al*., 1998; Tajima *et al*., 2001), often resulting in decreased ruminal pH. Ruminal pH is critical to normal function of the rumen, and monitoring ruminal pH is crucial in the diagnosis of ruminal acidosis (Enemark *et al*., 2002). Ruminal pH also has a key role in ruminal motility, as well as production and absorption of organic acids. If the amount of available substrate within the rumen of feedlot cattle is not excessive and absorption of organic acids across the epithelial wall is equivalent to production, ruminal pH will usually remain above 5.5 (Nagaraja and Titgemeyer, 2007). Ruminal pH ranging from 5.0 to 5.6 is generally referred to as subacute or chronic acidosis; and a ruminal pH below 5.0 is considered acute acidosis (Britton and Stock, 1989; Owens et al., 1998; Krause and Oetzel, 2006). Feed intake depression occurs when the rumen pH

falls below 5.5 (Fulton et al., 1979). Ruminal acidosis has been shown in past research to have detrimental effects on feed intake. Subacute acidosis can be characterized in feedlot cattle by a reduced and erratic feed intake (Fulton, 1979). Cattle also may experience decreased gain and efficiency (Stock *et al*. 1990). Considering these factors, it is important to monitor feed intake closely.

Following initial inoculation and introduction of the concentrate diet on the morning of d 2, ruminal pH decreased in all steers ($P < 0.05$). By 8 h post-feeding, steers that received the 100 mL dose had a mean pH of 5.0, which by the next morning had increased to 5.5. By 24 h post-feeding, steers in the placebo group had a lower ruminal pH compared to steers dosed with live inoculum ($P < 0.05$). The lower ruminal pH can be explained by the higher 24 h DMI and resultant increased acid production by steers in the placebo group ($P < 0.05$). Ruminal pH and DMI for d 2 are shown in Figure 2-2.

Compared to the placebo group, cattle administered *M. elsdenii* maintained higher ruminal pH on d 3 ($P < 0.05$). The placebo group had a mean ruminal pH of 5.26 at 0800 h on the morning of d 3, which was lower than the 8 h post-feeding level on the previous day. In fact, 2 steers from the placebo group had ruminal pH below 5.0 during this sampling period. Steers dosed with live cultures maintained a mean ruminal pH above 5.6 the entire sampling period on d 3. Steers assigned to the placebo group had a mean ruminal pH of 5.38 at 8 h post-feeding. In this case, there was a higher concentration of ruminal lactate compared to steers dosed with live inoculum ($P < 0.05$). No differences were noted among treatment groups for DMI on d 3. The combined effects of ruminal pH below 5.0 and excessive ruminal lactate concentrations was most likely detrimental to normal rumen function during this sampling period. Consequently, it

can be postulated that the placebo group experienced a metabolic insult during the entire sampling period for d 3. The values for d 3 ruminal pH and DMI are shown in Figure 2-2.

On the morning of d 4, mean ruminal pH in all of the steers remained above 5.5, with steers in the placebo group being numerically lower compared to steers dosed with live inoculum until 6 h post-feeding. No differences were noted among treatment groups relative to DMI. Ruminal pH values and DMI for d 4 are shown in Figure 2-2.

On d 5, all steers had a mean pH that fell within a range of 5.5 to 6.1. No differences among treatment groups relative to ruminal pH and DMI were apparent for d 5. Ruminal pH and DMI values for d 5 are shown in Figure 2-2.

On d 7, following step-up to the 80% concentrate diet, it was expected that ruminal pH would decrease in response to the diet change. Steers in the placebo group had a higher 24 h DMI compared to steers dosed with live cultures ($P < 0.05$). Among all treatments, there were no differences relative to ruminal pH for d 7. Day 7 pH and DMI data are shown in Figure 2-3.

After the final step-up to a steam-flaked corn-based finishing diet consisting of 94% concentrate on d 12, it is conceivable that another insult occurred. Given that the steers on this experiment were stepped up to a final finishing diet in a relatively brief amount of time, an insult would not be unexpected. By 8 h post-feeding, mean ruminal pH in all steers had fallen below 5.5. The pH and DMI relationships are illustrated in Figure 2-3.

On d 16, steers were again fasted for 24 hours. Having little carbohydrate substrate remaining in the rumen for normal microbial fermentation, mean ruminal pH levels prior to re-alimentation on the morning of d 17 were above 7.0 for all steers. As expected, steers gorged themselves, and ruminal pH of all steers decreased precipitously. At the end of the sampling period (8 h post-feeding), steers in the 1000 mL dose group were the only cattle with

pH of at least 5.5. Steers in the placebo group had higher 24-h DMI on d 17 ($P < 0.05$). Figure 2-3 displays ruminal pH for d 17.

Day 18 was not originally planned in the trial protocol, but we decided to take a ruminal pH at 4-h post-feeding. Ruminal pH levels for all steers fell below 5.5, with no differences among treatments in DMI (Figure 2-4).

Four h post-feeding on d 24, mean ruminal pH values were highest for the placebo group, but all treatment groups were below 5.5 during the sampling time. Steers in the placebo group had higher 24-h DMI on d 24 ($P < 0.05$). Day 24 values are illustrated in Figure 2-4.

On d 31, also a 4-h post-feeding sampling time, no differences in ruminal pH levels were noted among treatments. Steers in the placebo group did have numerically higher pH, which may be explained by their lower DMI. Steers dosed with live *M. elsdenii* had a higher 24 h DMI on d 31 ($P < 0.05$). Figure 2-4 gives the DMI and ruminal pH relationships for d 31.

At the last sampling, d 42, all steers had ruminal pH levels below 5.5, and cattle in the placebo group more feed over 24-h ($P < 0.05$; Figure 2-4).

Final statistical analyses for pH revealed that there were differences among treatments ($P < 0.05$). Steers receiving the live doses of *M. elsdenii* maintained higher ruminal pH levels compared to the placebo group during the first 24 h post-feeding ($P < 0.05$). These differences occurred during the initial grain-challenge. No persistence of response to dosing with *M. elsdenii* was noted by 48 h (d 3) after inoculation and diet changes occurred. Curtailing an initial insult by dosing cattle with *M. elsdenii* could prove useful if administered immediately before diet changes. Dose size did not appear to be a factor impacting response. The initial introduction of highly fermentable substrates such as steam-flaked corn into the rumen is a common occurrence in newly arrived feedlot cattle. It is conceivable that all cattle during this

study experienced ruminal insults based on pH and fluctuation in DMI. Differences among a population of cattle relative to their individual abilities to handle ruminal insults are important to consider. One of the most critical time points in feeding cattle is during the introduction of these highly fermentable diets. These data reveal that the steers dosed with live cultures of *M. elsdenii* strain NCIMB 41125 maintained ruminal pH levels higher than that of the placebo group during the crucial period after introduction of grain into the diets of feedlot cattle ($P < 0.05$). Exploiting such characteristics of the investigated strain of *M. elsdenii* could be a very useful tool in the feedlot industry if fed prior to introduction of concentrate-based feeds.

Ruminal VFA and Lactate Concentrations

Organic acids are the products of normal microbial digestion of substrates within the rumen. Newly arrived feedlot cattle that have not previously consumed grain-based diets may experience substantial changes in ruminal microbial populations. Introduction to finishing diets is usually accomplished gradually by feeding increasingly greater proportions of concentrate feeds, thus allowing ruminal bacterial populations to shift from predominantly cellulolytic species to amylolytic species. Steers on the current experiment were abruptly switched from a forage-based diet to a 66% concentrate diet following a 24-h fast.

Ruminal concentrations of volatile fatty acids were altered in response to the diet change ($P < 0.05$). Following introduction of grain in the diet, ruminal acetate increased, with no differences among treatments (Figures 2-5 and 2-6). Ruminal propionate levels increased in response to the diet change (Figures 2-7 and 2-8). On d 3, ruminal acetate to propionate ratios were higher in the placebo group ($P = 0.05$) and are shown in Figures 2-9 and 2-10. In response to diet change, ruminal production of butyrate increased, without differences among the treatments during the experiment (Figures 2-11 and 2-12). Isobutyrate production responded to

diet change, but no differences were noted during the study among treatments (Figures 2-13 and 2-14). Isovalerate was higher on d 17 in steers dosed with live culture of *M. elsdenii* compared to the placebo group ($P < 0.05$; Figures 2-15 and 2-16). Ruminal valerate concentrations were higher on d 3, 24, 31, and 42 in cattle dosed with live *M. elsdenii* compared to the placebo group ($P < 0.05$; Figures 2-17 and 2-18). Total VFA concentrations were lower on d 3 for the placebo group compared to cattle that received live cultures of NCIMB 41125 (Figures 2-19 and 2-20).

High acetate to propionate ratios are generally viewed as undesirable. Propionic acid is the most preferred ruminal fermentation product because of its gluconeogenic properties. Since *M. elsdenii* ferments lactate into propionate via the acrylate biochemical pathway (Counotte *et al*., 1981), it is an ecologically important bacteria in finishing cattle (Hino *et al*., 1994). On d 3, steers in the placebo group had extremely high acetate to propionate ratios. In the case of the placebo group in our study, several inferences might be made about the increased acetate to propionate ratio we discovered. Most likely, the low ruminal pH levels among the placebo group on d 3 had detrimental effects on ruminal fermentation. In support of this postulation, it is useful to consider the low concentration of total volatile fatty acids on the day in question. When feedlot cattle become affected by acute acidosis, total VFA concentration usually increases during onset and declines substantially with progression due to destruction of the normal ruminal microflora and the deluge of fluids entering the rumen in an attempt to regulate osmolality (Huber, 1976). Increased production of VFA can be attributed to proliferation of *lactobacilli*, and a decrease in fermentation is the result of ruminal pH falling below a range that is supportive of LUB activity (Therion *et al*., 1982). In subacute acidosis, the combined effects of overproduction and decreased absorption cause an accumulation of VFA in the rumen and a resulting decrease in ruminal pH to below 5.6.

Branched chain VFA such as isovalerate are produced in the rumen due to the catabolism of amino acids (Andries et al., 1987). *M. elsdenii* is most noted for its ability to utilize lactate, but may also ferment amino acids (Elsden et al., 1956). Catabolism of amino acids by an organism known to be an efficient lactate-utilizer in an environment characterized by high levels of lactate, such as in the rumen of grain-fed cattle, is possible. Based on the fact that amino acid catabolism is considered a minor source of energy for *M. elsdenii*, amino acids are most likely catabolized by other organisms in the rumen (Wallace, 1986).

Concerning the valerate concentrations, it is interesting to know that *M. elsdenii* is a producer of this organic acid (Hungate, 1966; Marounek *et al*., 1989). Higher ruminal populations of *M. elsdenii* in steers dosed with live cultures may be related to an increased concentration of valeric acid.

Ruminal lactate concentrations are shown in Figures 2-21 and 2-22. Lactate concentrations in the rumen increased in response to the diet change ($P < 0.05$). Concentrations were lower for cattle that were dosed with *M. elsdenii* compared to the placebo group ($P < 0.05$). On d 3, ruminal lactate concentrations peaked for the placebo group and remained higher than those steers dosed with live *M. elsdenii* ($P < 0.05$). High lactate concentrations in the cattle were expected. Even though ruminal lactic acid production increases with the incidence of subacute acidosis, LUB normally prevent any substantial accumulation (Goad *et al*., 1998). If ruminal pH approaches and remains at 5.0 or less for a significant amount of time, lactate accumulates because ruminal conditions are inhibitory to LUB and subacute acidosis may advance to lactic acidosis. Unfortunately, we do not know an exact amount of time needed for progression of this metabolic disorder. Lactate can be present in the rumen of cattle being fed grain in two different isomeric forms. The optical isomers of lactate are L (+) lactate and D (-) lactate. L(+) is the

predominant isomer of the two, and D(-) usually increases as ruminal pH decreases (Giesecke and Stangassinger, 1980). *M. elsdenii* has the ability to take advantage of either form of lactate through the use of the enzyme racemase (Asanuma and Hino, 2002). Cattle dosed with live cultures of *M. elsdenii* strain NCIMB 41125 were able to maintain ruminal lactate concentrations lower than the placebo group ($P < 0.05$). The fact that steers dosed with live cultures maintained lower lactic acid levels within their rumens was expected, as *M. elsdenii* is a known to be an important lactic acid utilizing species. The differences among treatments were most evident at 24 h post-feeding (24 h after the initial carbohydrate challenge). Referring back to ruminal pH levels of steers in our study, we can relate high lactate concentrations in the placebo group to their low ruminal pH levels experienced in the first 24 h after introduction to the concentrate diet. No persistence of response to dosing was evident by 48 h (d 3) post-inoculation. Ruminal lactate concentrations were similar among all treatments after 48 h. Dosing cattle with *M. elsdenii* would most likely be effective in reducing incidences of lactic acidosis following an initial diet change. Since the largest insult to feedlot cattle occurs during the initial introduction to grain, our results are supportive of the hypothesis that dosing newly arrived feedlot cattle with *M. elsdenii* strain NCIMB 41125 is a possible way to reduce accumulation of lactic acid within the rumen.

Conclusions

Our results show that steers dosed with live *M. elsdenii* strain NCIMB 51125 maintained a higher ruminal pH than steers in the placebo group. Furthermore, results of the study illustrate that dosing cattle with *M. elsdenii* strain 41125 is a viable means of reducing the concentrations of lactic acid in cattle being fed grain-based diets. Dosing cattle with *M. elsdenii* before introduction of a concentrate diet may help to prevent the accumulation of lactic acid and in so

doing avoid severe depressions in ruminal pH. Dosing newly-arrived feedlot cattle with *M. elsdenii* strain NCIMB 41125 may be useful for managing acidosis.

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Table 2-1. Composition of experimental diets fed to steers intraruminally dosed with a placebo or live cultures containing 1.62×10^8 cfu/mL of *Megasphaera elsdenii* strain NCIMB 41125.

	Amount of Concentrate		
	66%	80%	94%
Ingredient, % of DM			
Steam-flaked corn	56.9	70.8	84.4
Ground alfalfa hay	33.2	19.4	5.8
Corn steep liquor	6.5	6.5	6.4
Vitamin-mineral premix[1]	3.4	3.4	3.3
Nutrient Composition			
CP, %	15.7	14.8	14.0
NE_m, Mcal/kg	1.87	2.05	2.20
NE_g, Mcal/kg	1.23	1.39	1.54
Ca, %	1.05	0.88	0.71
P, %	0.36	0.37	0.38

[1]Formulated to provide 0.1 mg/kg Co, 10 mg/kg Cu, 0.6 mg/kg I, 60 mg/kg Mn, 0.25 mg/kg Se, 60 mg/kg Zn, and 2205 IU/kg of vitamin A.

Figure 2-1. Timeline of sampling times and dietary changes.

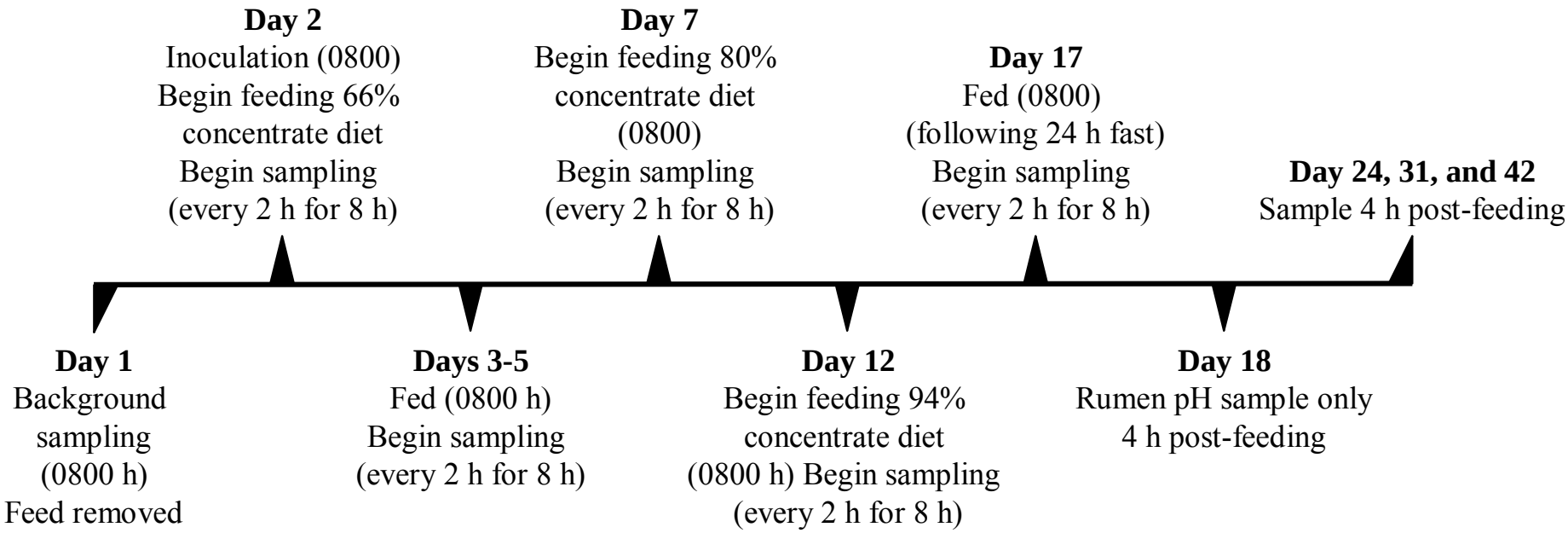
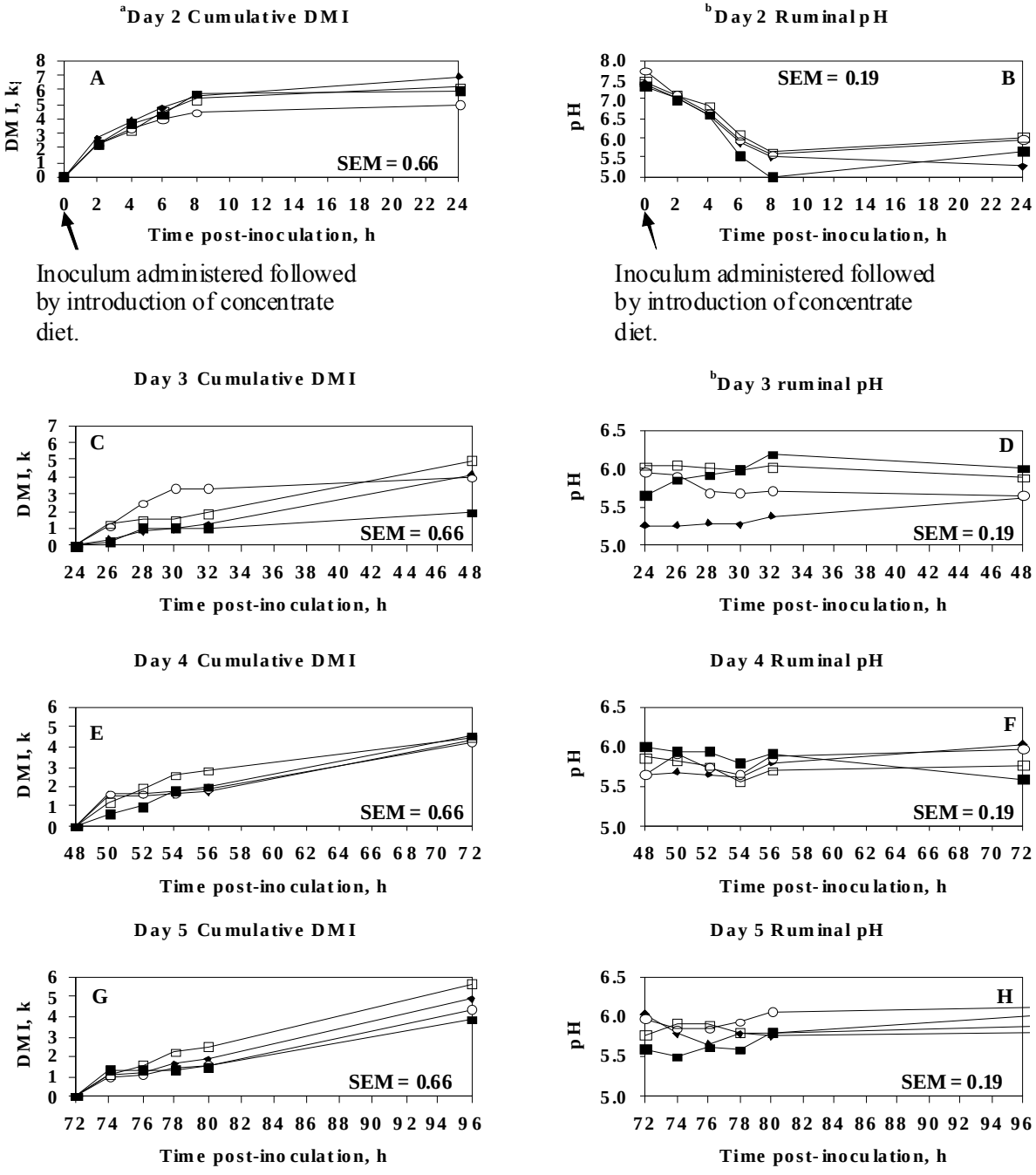


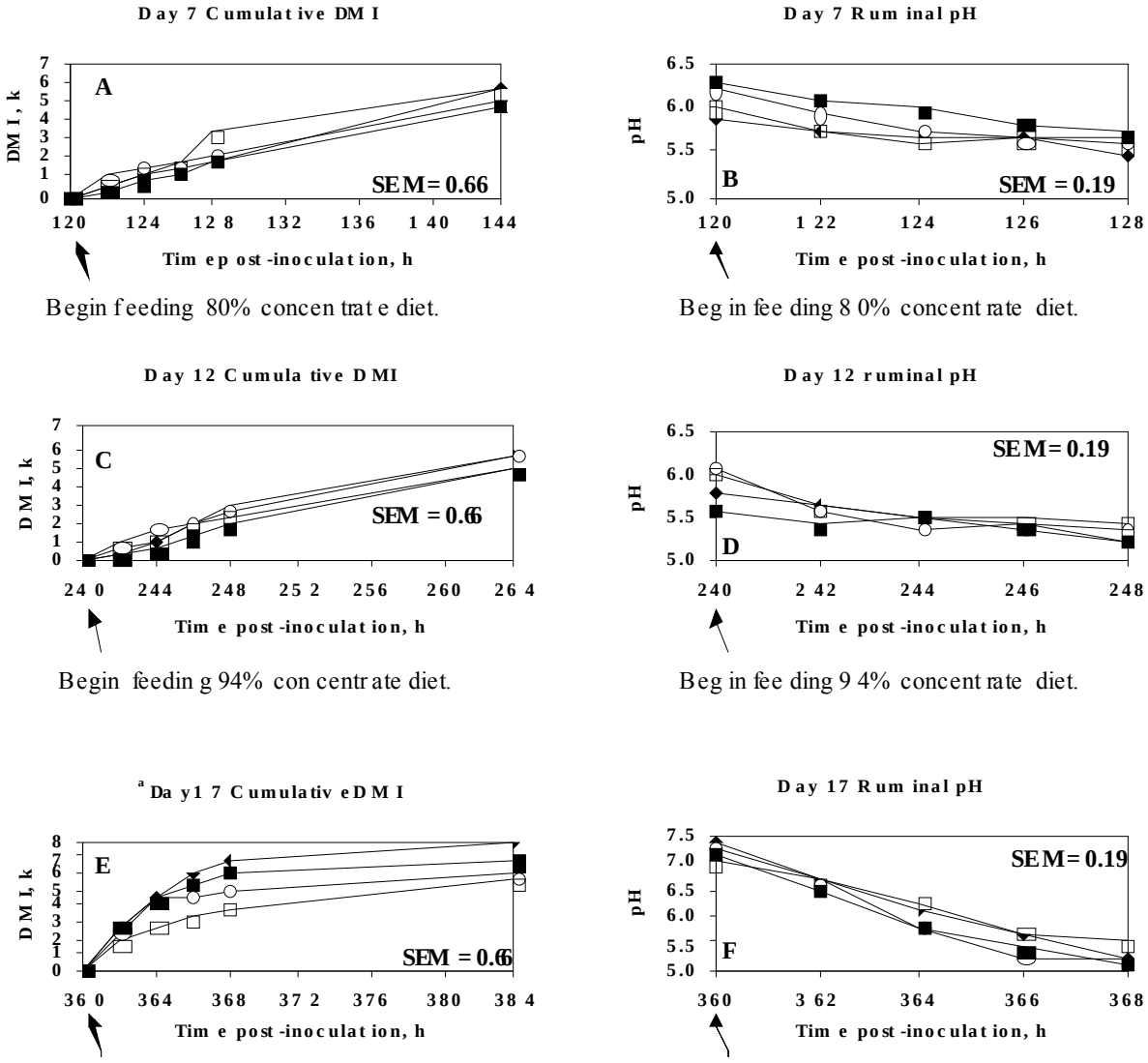
Figure 2-2 (A through H). Day 2 through 5 cumulative DMI and ruminal pH of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125. (Placebo —◆—, 10 mL —○—, 100 mL —■— , 1000 mL —□—)



[a]Higher 24-h cumulative DMI among the steers dosed with placebo vs average of *M. elsdenii* ($P < 0.05$).

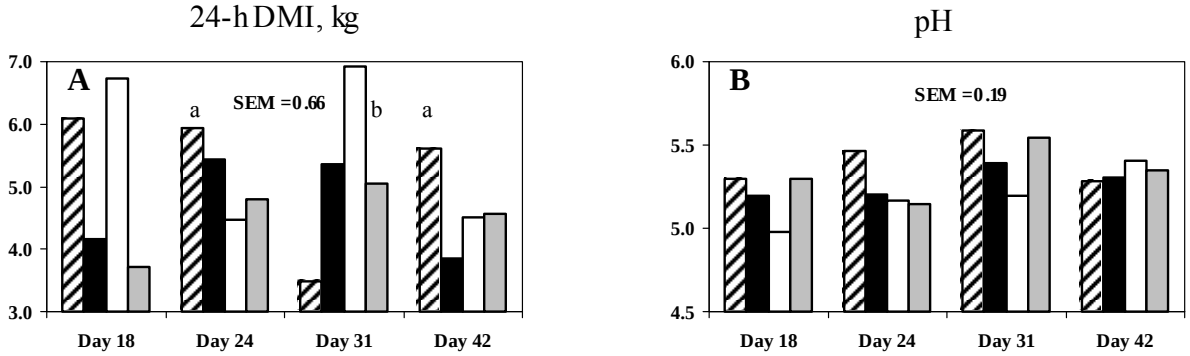
[b]Higher ruminal pH among the average of steers dosed with *M. elsdenii* vs placebo ($P < 0.05$).

Figure 2-3 (A through F). Day 7 through 17 cumulative DMI and ruminal pH of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125. (Placebo —◆—, 10 mL —○—, 100 mL —■— , 1000 mL —□—)



[a]Higher 24-h cumulative DMI among the steers dosed with placebo vs average of *M. elsdenii* ($P < 0.05$).

Figure 2-4 (A and B). Day 18, 24, 31, and 42 cumulative DMI and ruminal pH of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo ▨ , 10 mL■ , 100 mL □ , 1000 mL ▩)



[a]Higher 24-h cumulative DMI among the steers dosed with placebo vs average of *M. elsdenii* ($P<0.05$).

[b]Higher 24-h cumulative DMI among the steers dosed with *M. elsdenii* vs. placebo ($P<0.05$).

Figure 2-5 (A through D). Day 2 through 17 ruminal acetate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo —◆—, 10 mL —○— , 100 mL —■—, 1000 mL —□—)

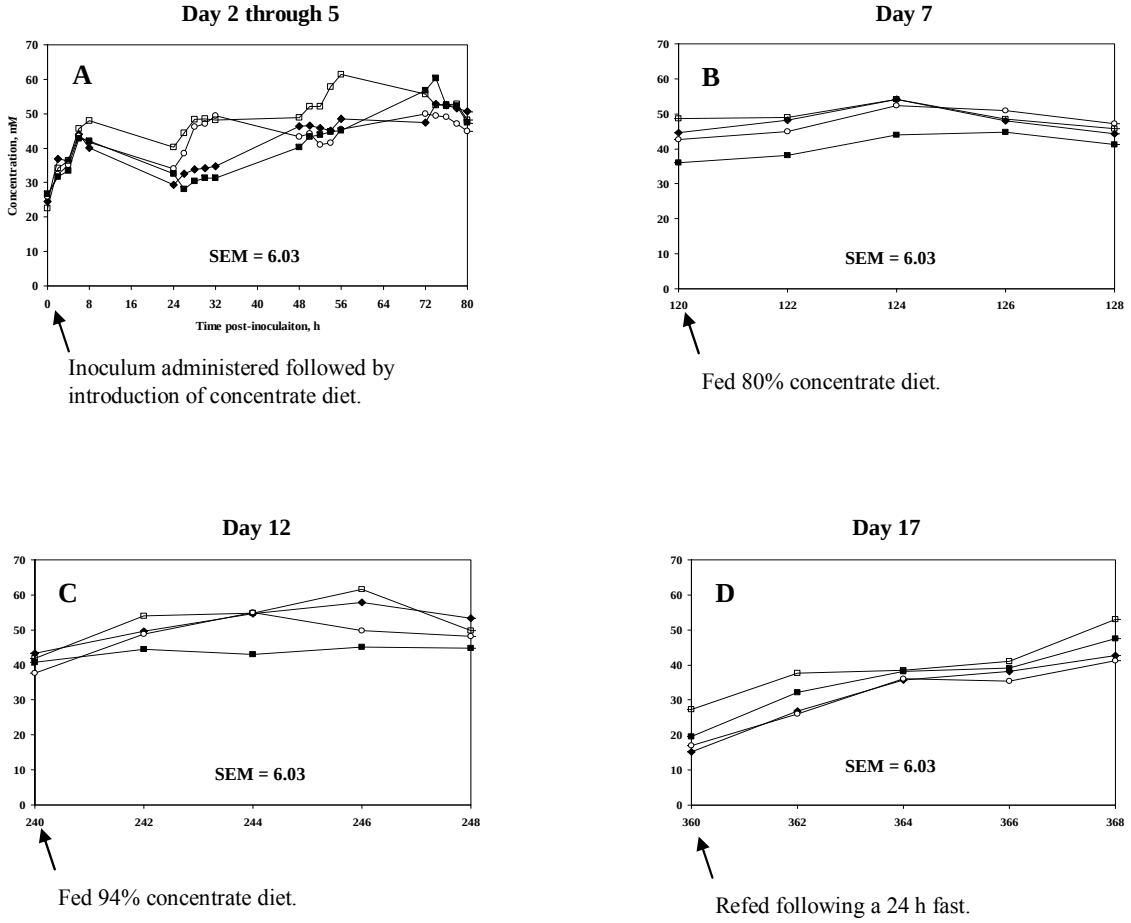


Figure 2-6. Day 24 through 42 ruminal acetate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo ▨ , 10 mL ■ , 100 mL □ , 1000 mL ▩)

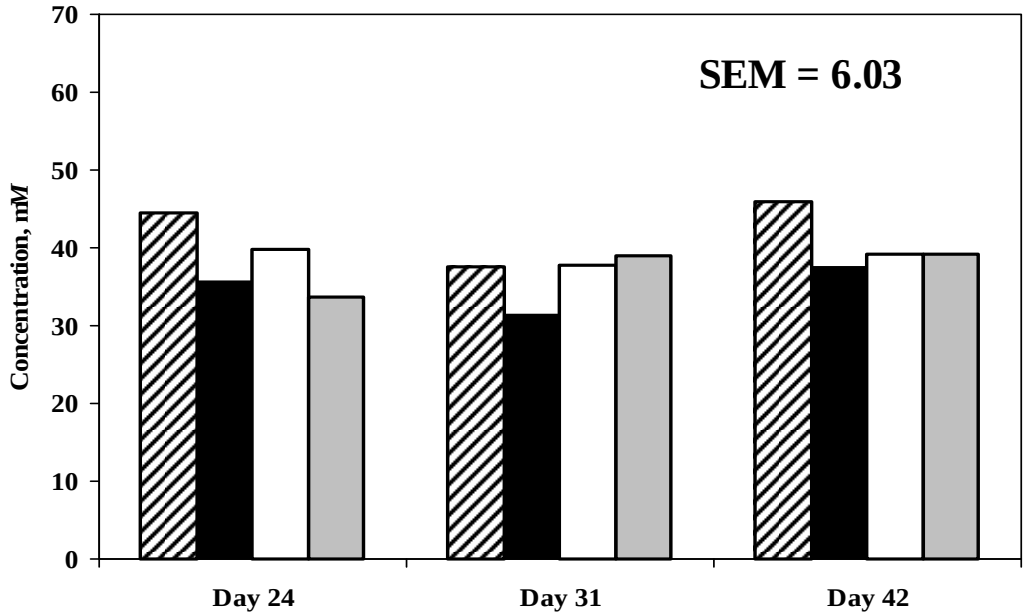


Figure 2-7 (A through D). Day 2 through 17 ruminal propionate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo —◆—, 10 mL —○—, 100 mL —■—, 1000 mL —□—)

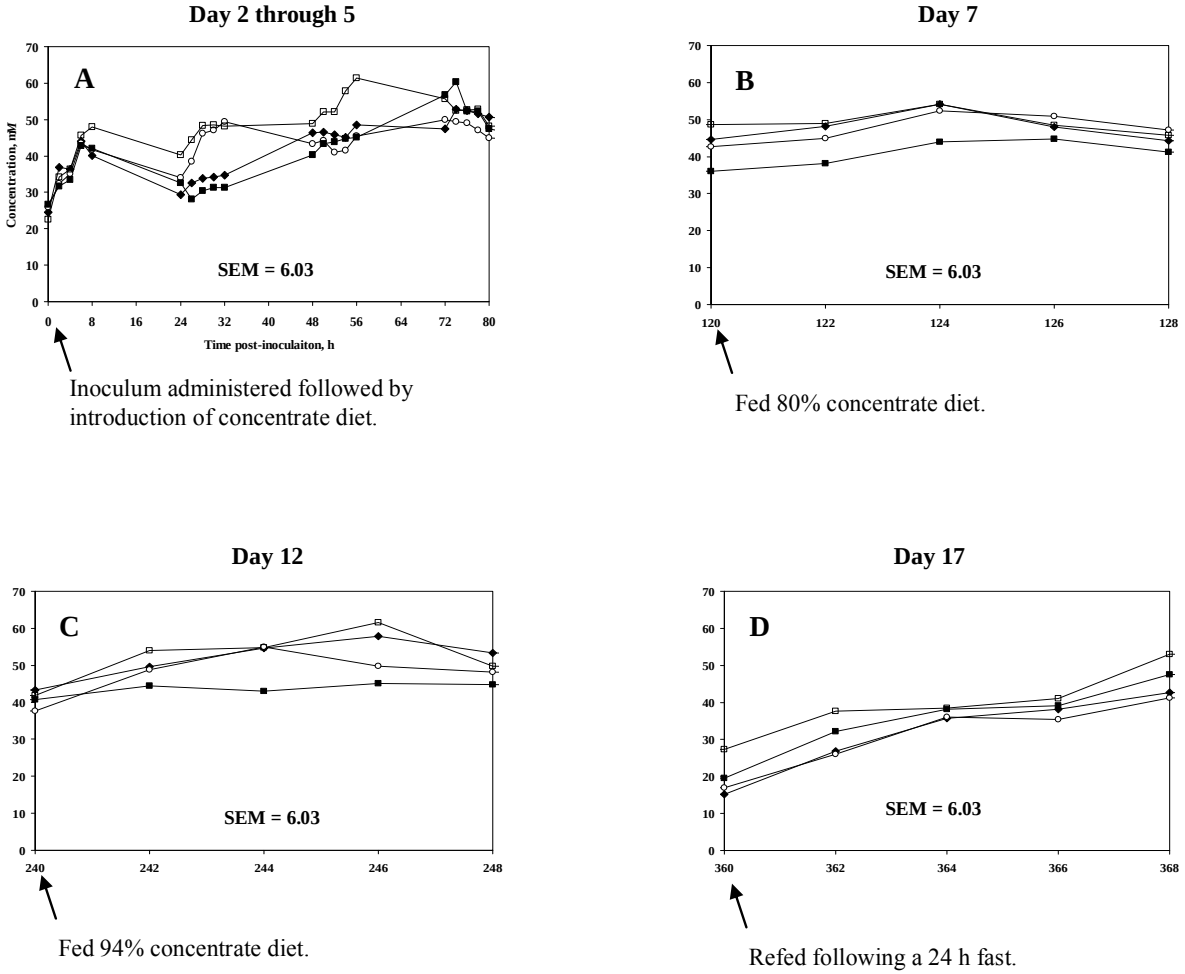


Figure 2-8. Day 24 through 42 ruminal propionate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo ▨, 10 mL ■, 100 mL □, 1000 mL ▩)

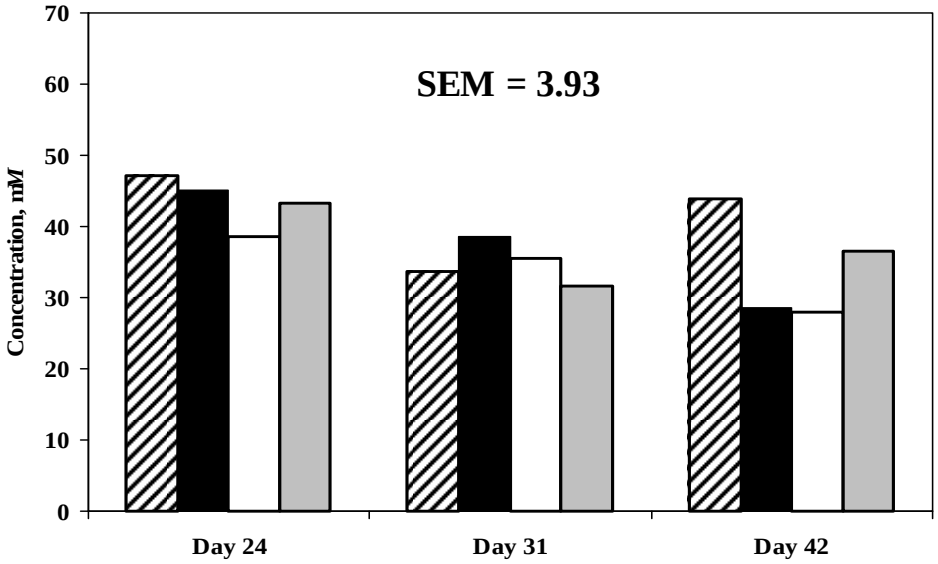
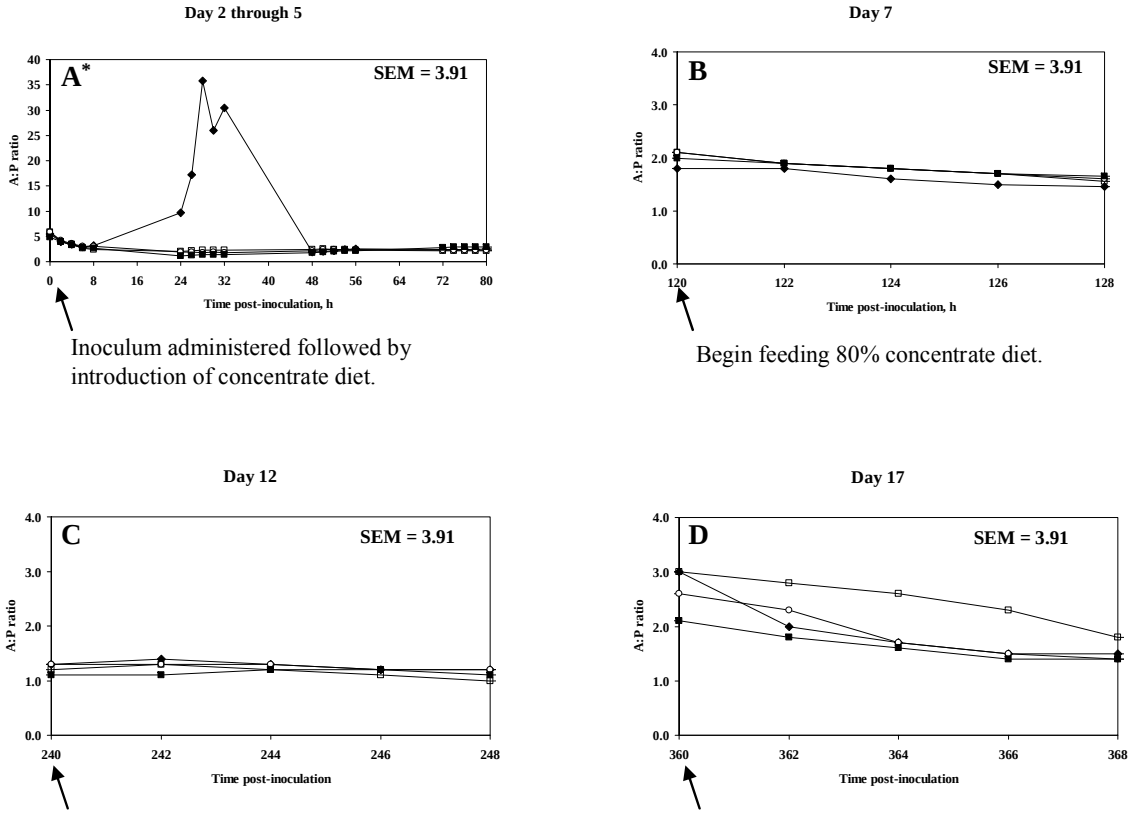


Figure 2-9 (A through D). Day 2 through 17 ruminal A:P ratios of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo —◆—, 10 mL —○—, 100 mL —■—, 1000 mL —□—)



A*: Lower ruminal A:P on d 3 among the average of steers dosed with *M. elsdenii* vs placebo ($P < 0.05$).

Figure 2-10.Day 24 through 42 ruminal A:P ratios of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo ▨, 10 mL ■, 100 mL □, 1000 mL▩)

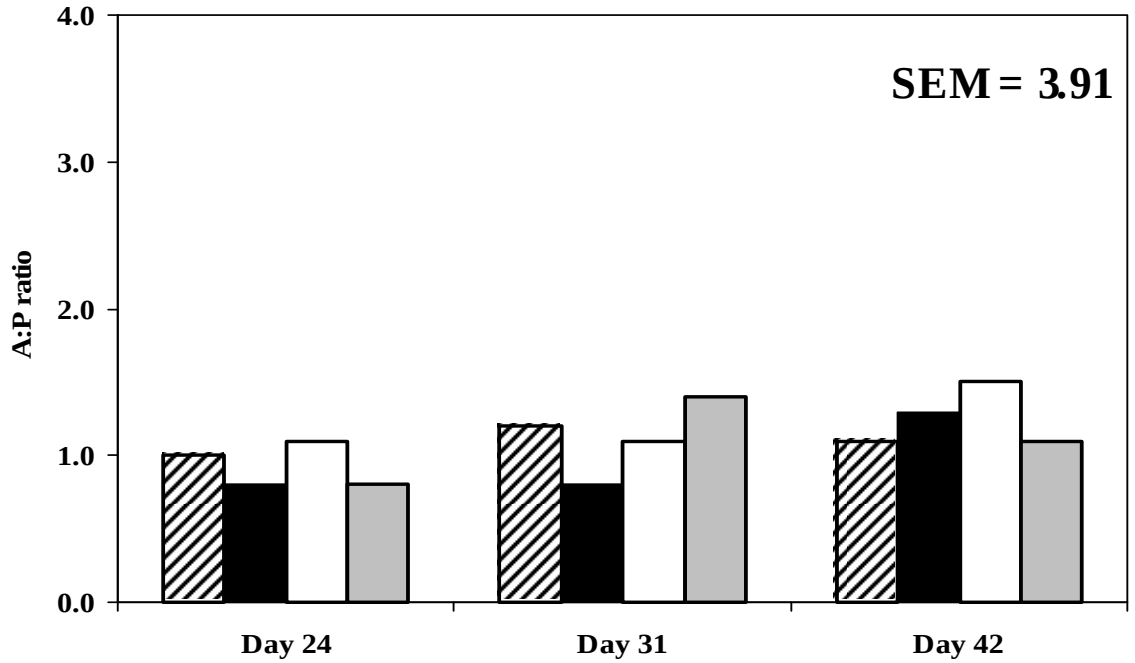


Figure 2-11 (A through D). Day 2 through 17 ruminal butyrate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo —◆—, 10 mL —○—, 100 mL —■— , 1000 mL —□—)

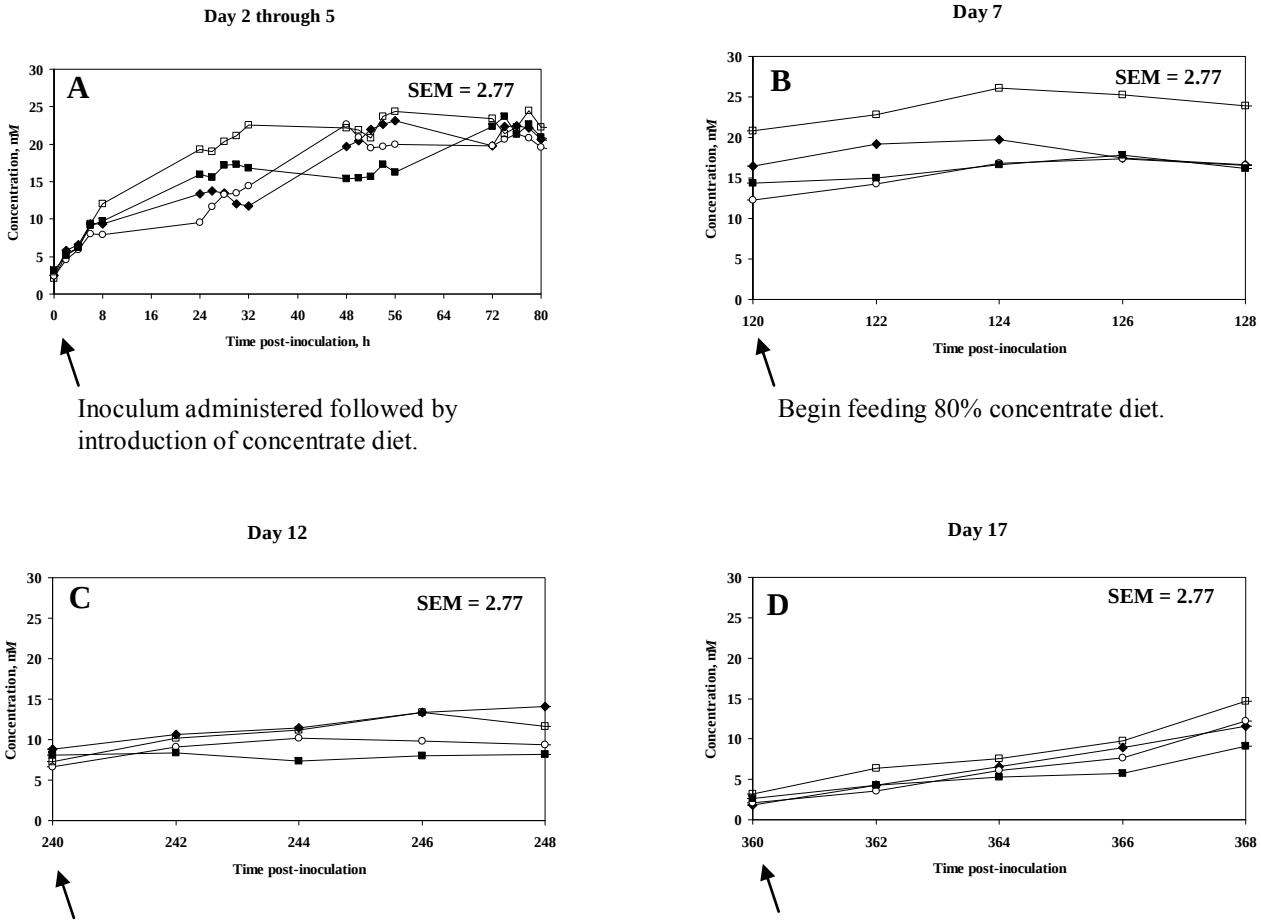


Figure 2-12. Day 24 through 42 ruminal butyrate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo ▨, 10 mL ■, 100 mL □, 1000 mL▩)

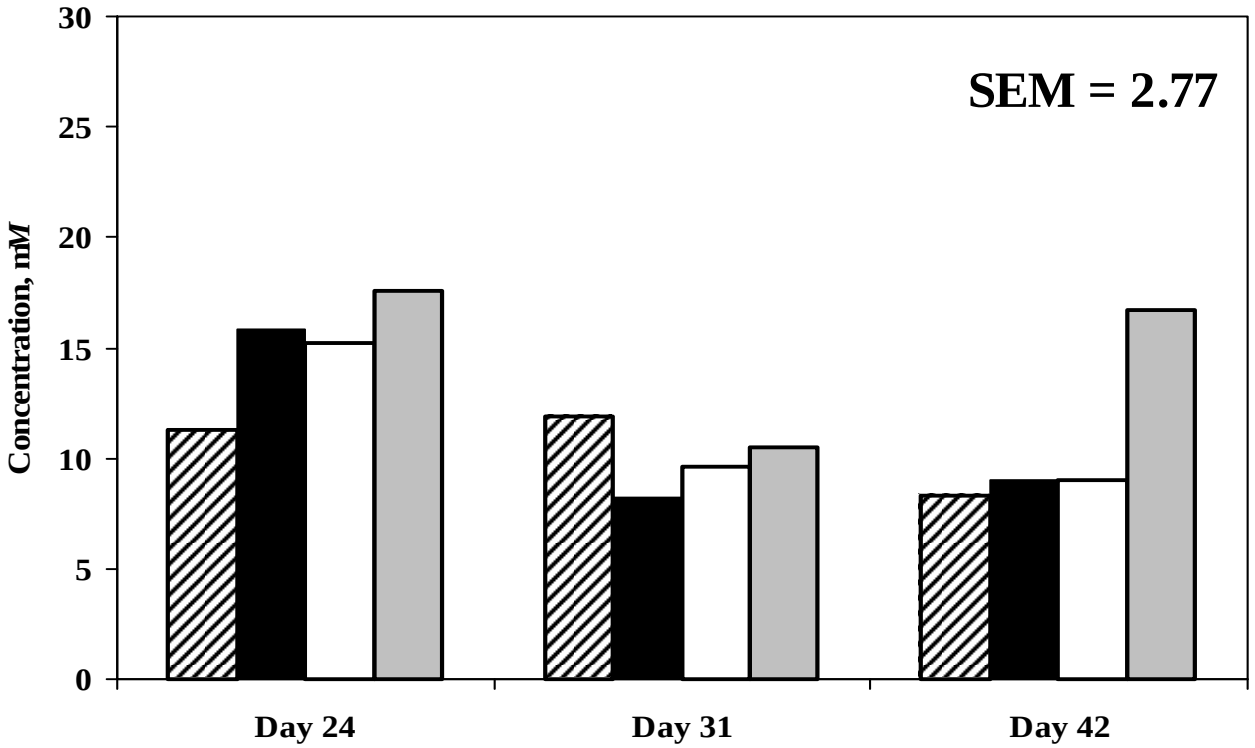


Figure 2-13 (A through D). Day 2 through 17 ruminal isobutyrate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo —◆— , 10 mL —○—, 100 mL —■— , 1000 mL —□—)

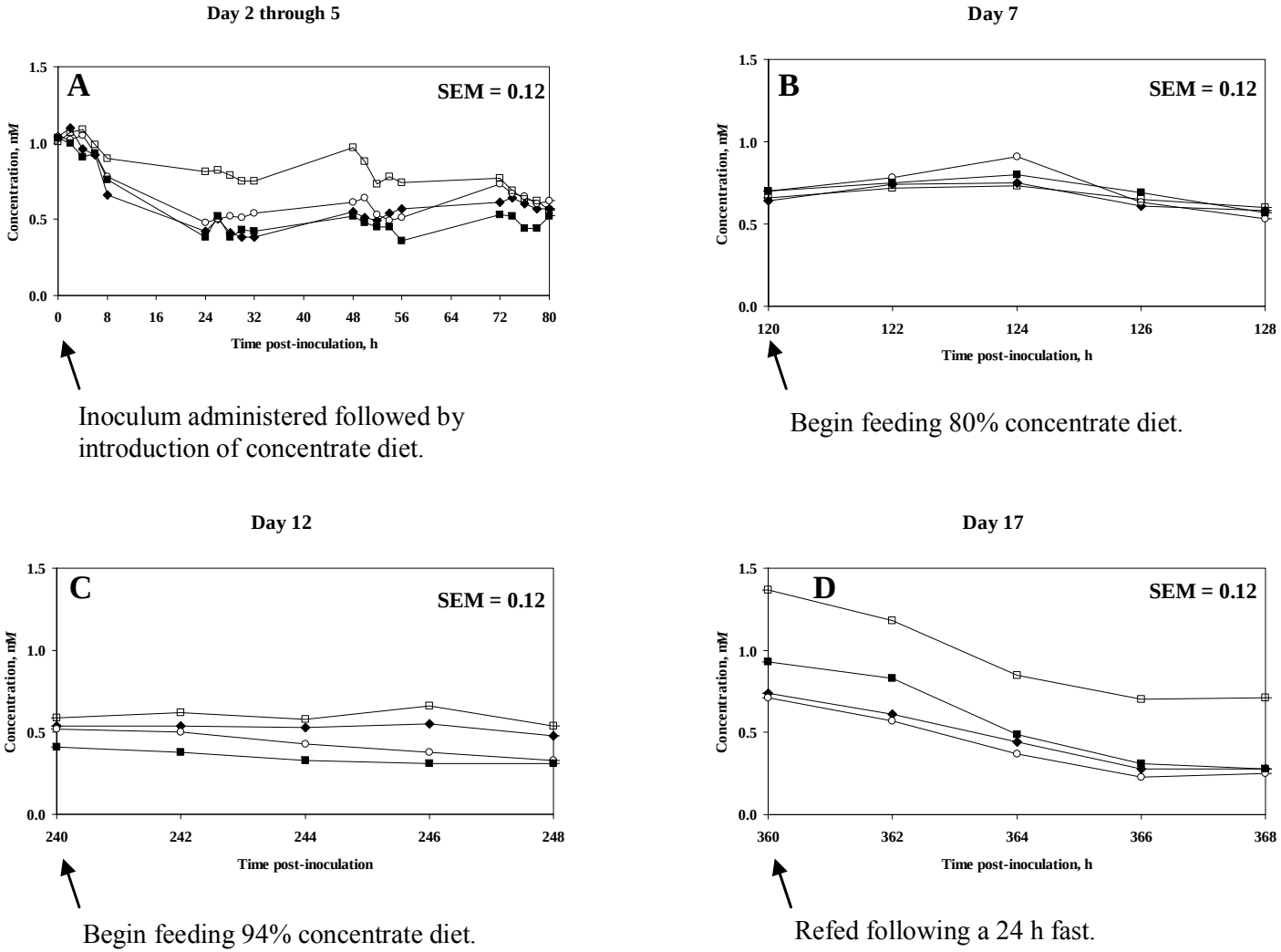


Figure 2-14. Day 24 through 42 ruminal isobutyrate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125
(Placebo ▨, 10 mL ■, 100 mL □, 1000 mL ▩)

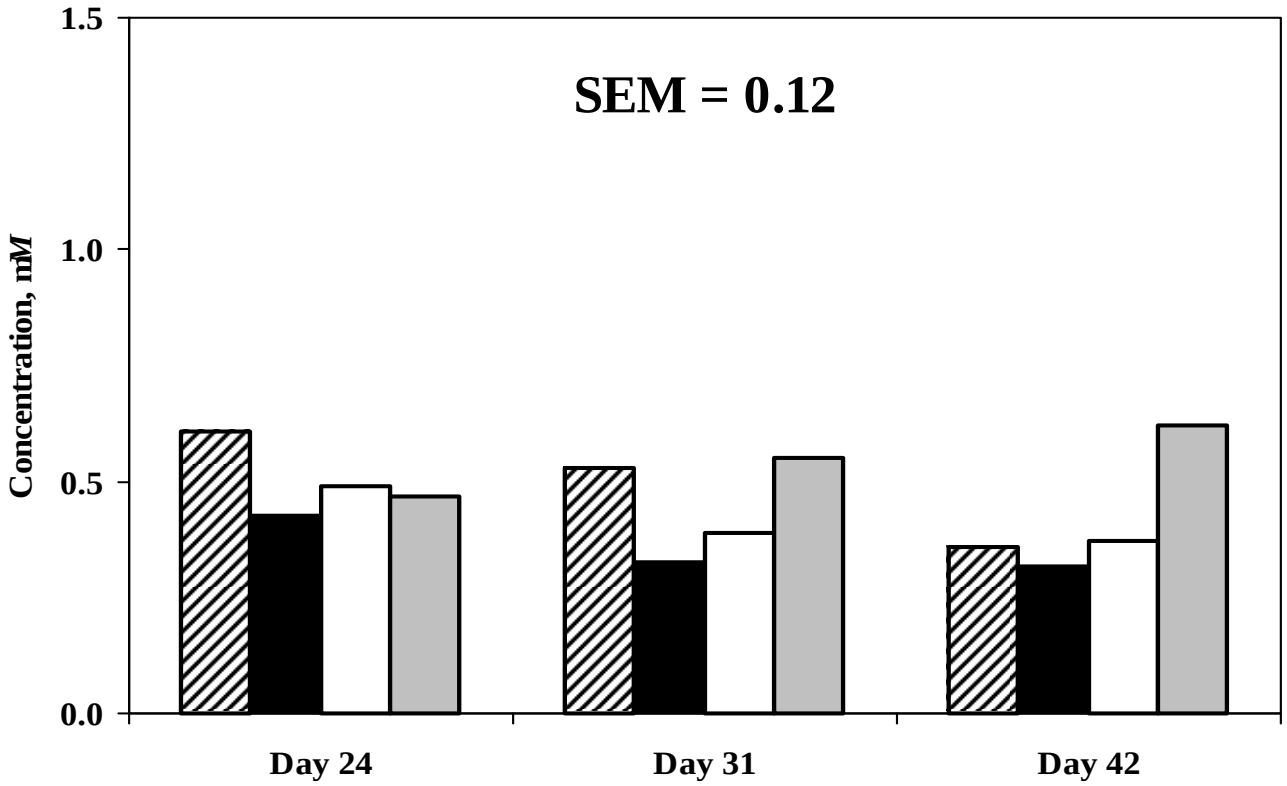
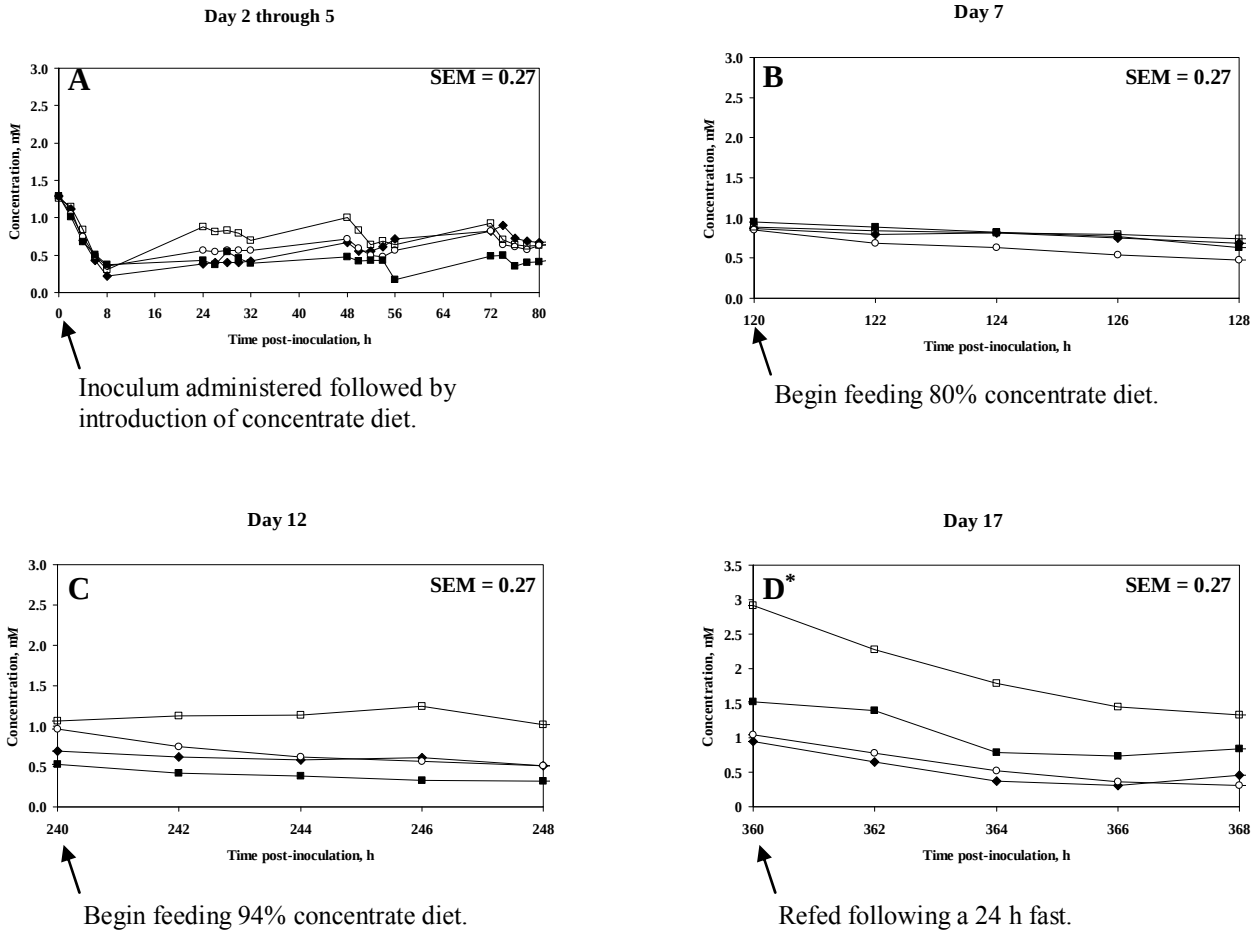


Figure 2-15 (A through D). Day 2 through 42 ruminal isovalerate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo —◆— , 10 mL —○—, 100 mL —■— , 1000 mL —□—)



D*: Higher ruminal isovalerate concentrations among the average of steers dosed with *M. elsdenii* vs placebo ($P < 0.05$).

Figure 2-16. Day 24 through 42 ruminal isovalerate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo ▨ , 10 mL ■, 100 mL □, 1000 mL ▩)

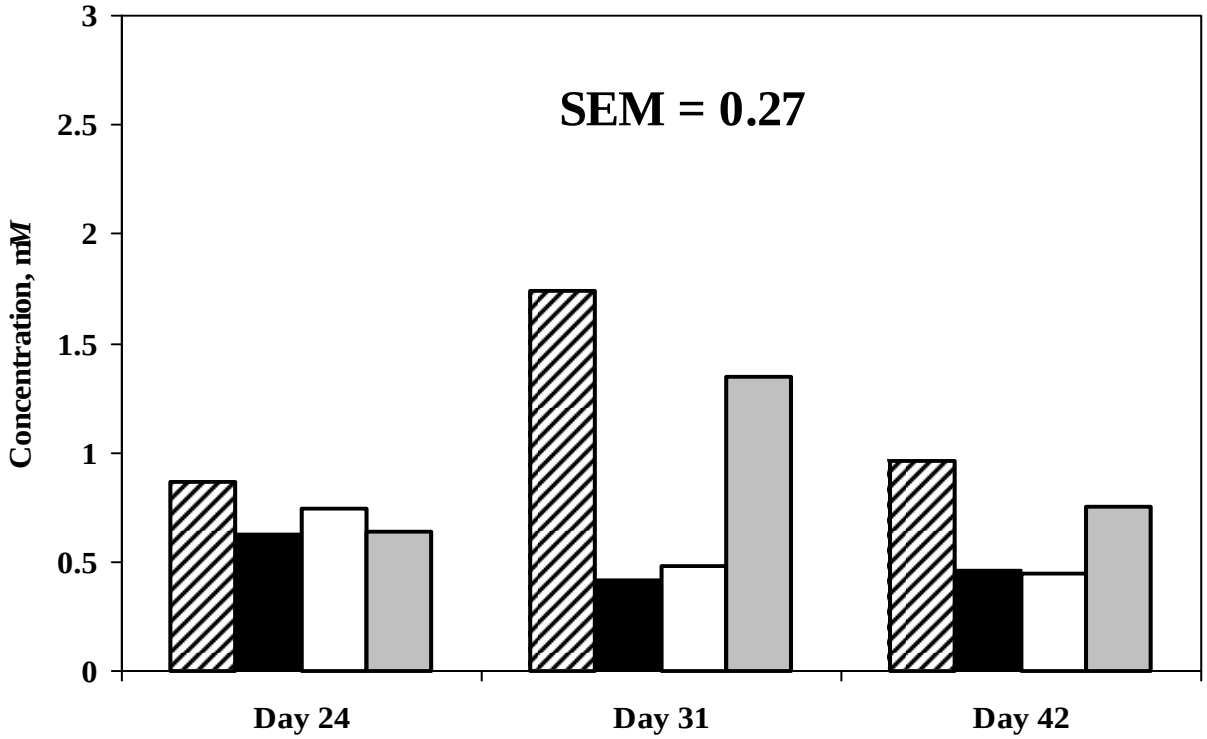
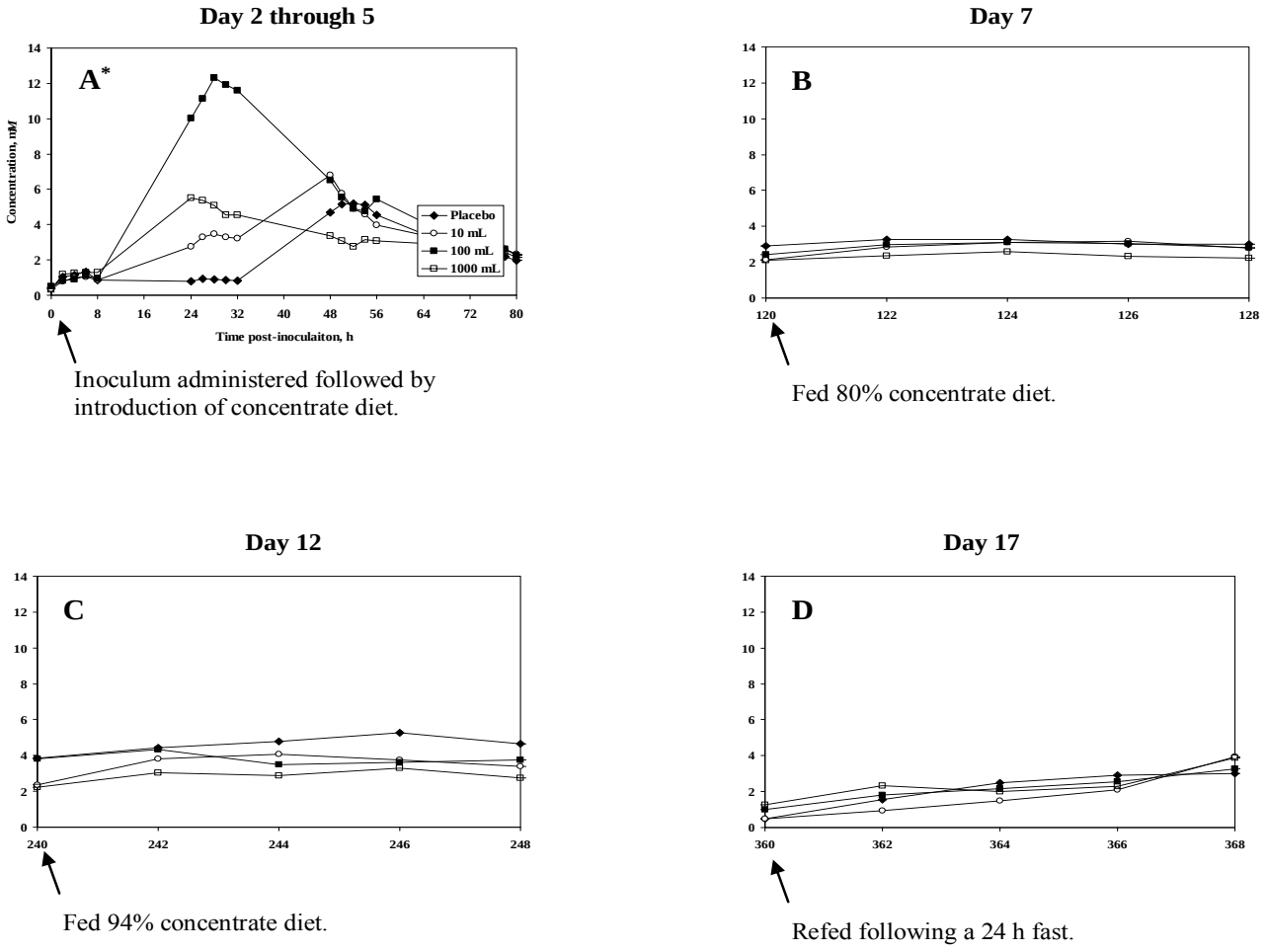
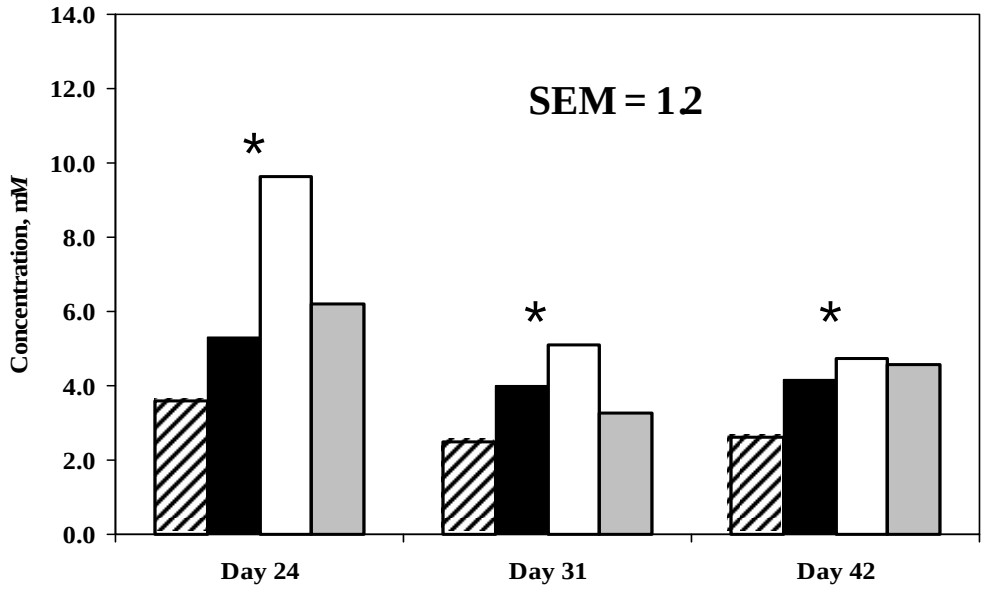


Figure 2-17 (A through D). Day 2 through 17 ruminal valerate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo —◆—, 10 mL —○—, 100 mL —■— , 1000 mL —□—)



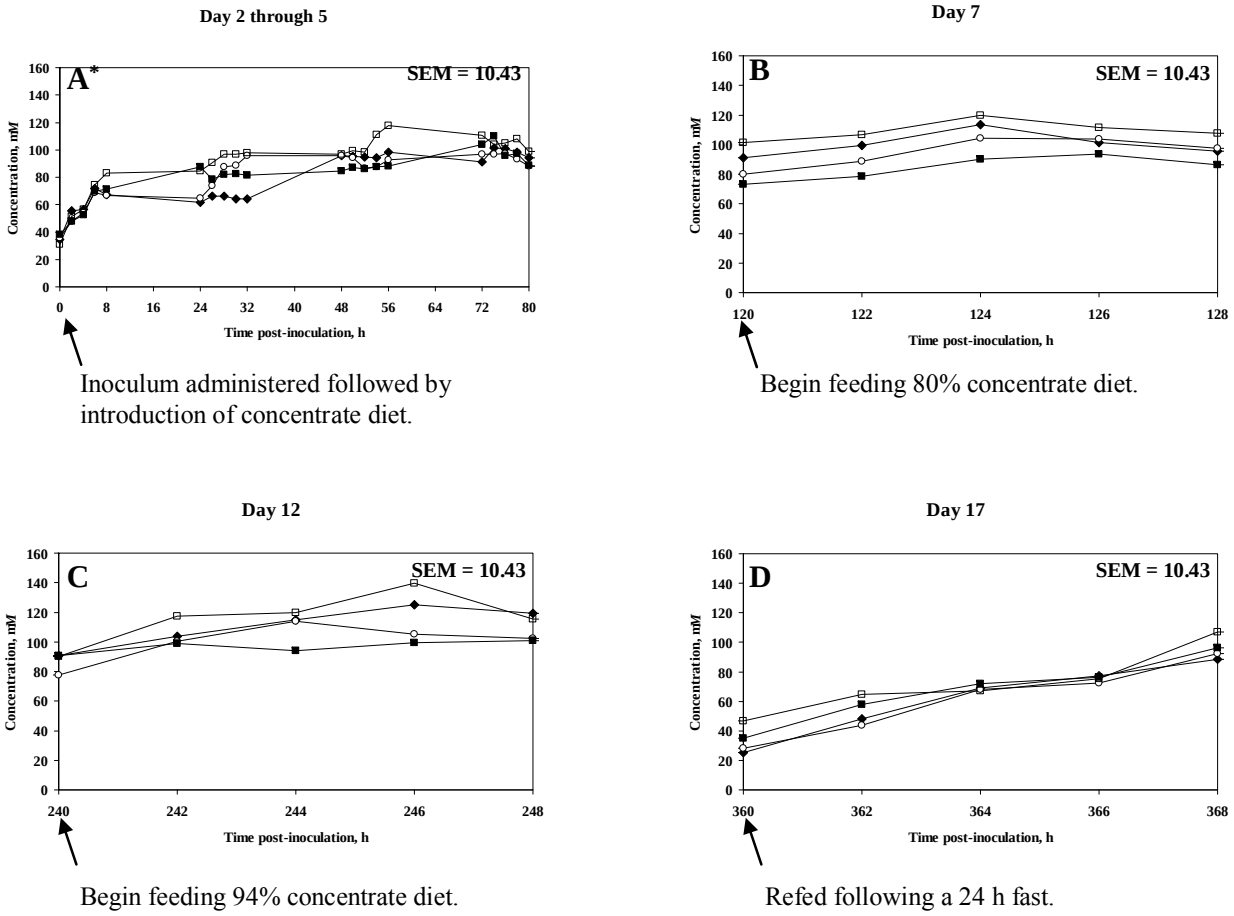
A*: Higher ruminal valerate concentrations on d 3 among the average of steers dosed with *M. elsdenii* vs placebo ($P < 0.05$).

Figure 2-18. Day 24 through 42 ruminal valerate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo ▨ , 10 mL ■ , 100 mL □ , 1000 mL ▩)



*Higher ruminal valerate concentrations on d 24, 31, and 42 among the average of steers dosed with *M. elsdenii* vs placebo ($P < 0.05$).

Figure 2-19 (A through D). Day 2 through 17 ruminal total VFA concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo —◆— , 10 mL —○— , 100 mL —■— , 1000 mL —□—)



***Lower ruminal total VFA concentration on d 3 (24-h post-inoculation) in placebo group ($P < 0.05$)**

Figure 2-20. Day 24 through 42 ruminal total VFA concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo ▨ , 10 mL ■ , 100 mL □ , 1000 mL ▩)

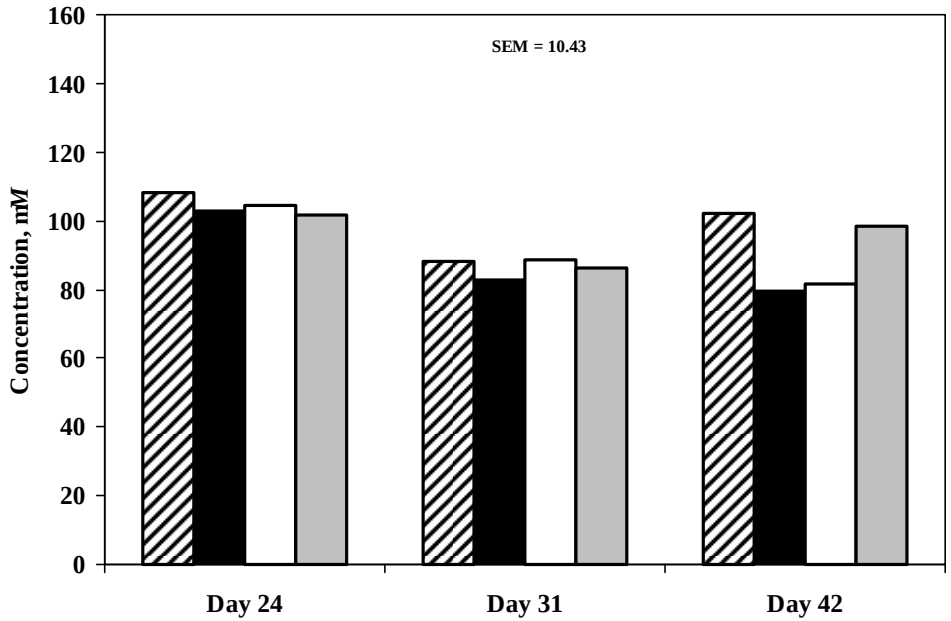
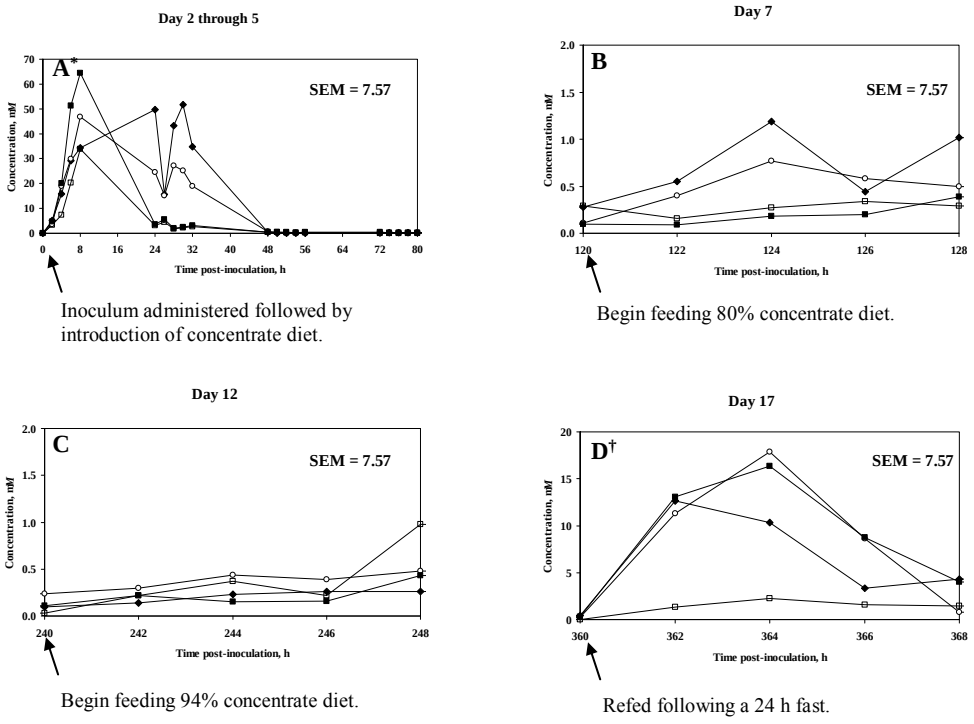


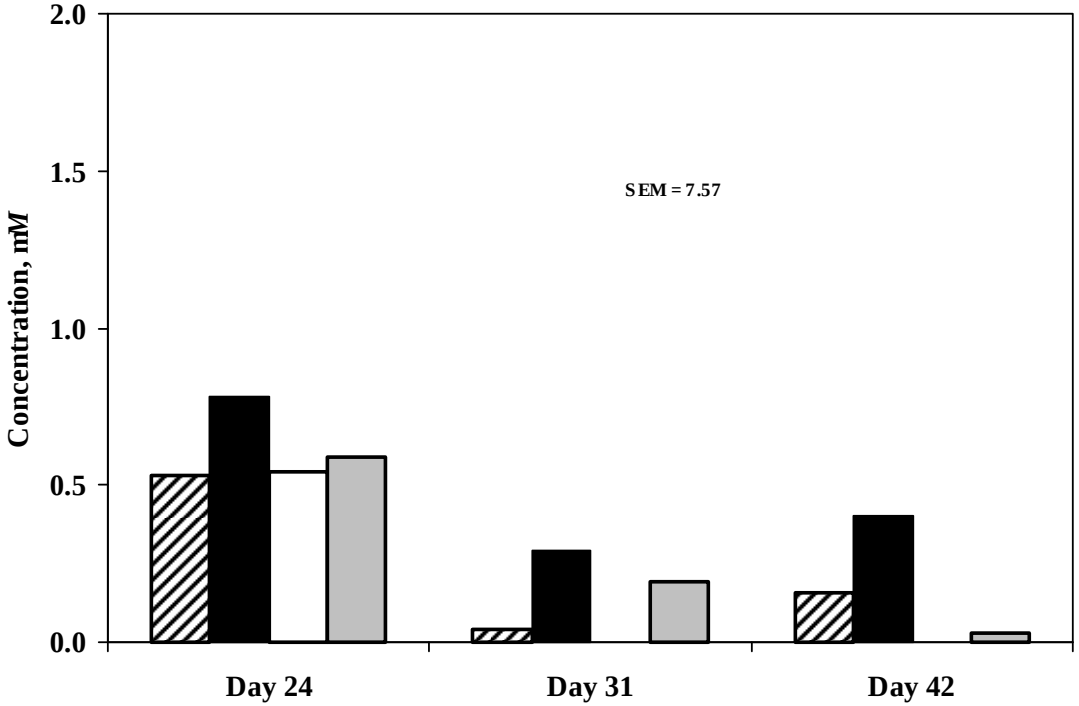
Figure 2-21 (A-D). Day 2 through 17 ruminal lactate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo —◆— , 10 mL —○—, 100 mL —■—, 1000 mL —□—)



A[*]: Lower ruminal lactate concentrations on d 3, among the average of steers dosed with *M. elsdenii* vs placebo ($P < 0.05$).

D[†]: Lower ruminal lactate concentrations, among the steers given the 1000 mL dose ($P < 0.05$).

2-22. Day 24 through 42 ruminal lactate concentrations of steers intraruminally dosed with a placebo or live cultures of *M. elsdenii* strain NCIMB 41125.
(Placebo ▨ , 10 mL■ , 100 mL□ , 1000 mL ▩)



CHAPTER 3 - Quantitative detection of bacterial genomes and *in vitro* studies to determine capacity for growth on lactate medium following intraruminal dosing of cattle with *Megasphaera elsdenii* strain NCIMB 41125

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This is contribution No. _____ from the Kansas Agricultural Experiment Station, Manhattan. KS.

Abstract

An experiment was conducted at Kansas State University Beef Cattle Research Center using Angus crossbred steers (n=20; initial BW=253 ± 24 kg) fitted with ruminal cannulas. Ruminal samples from steers were used to 1) quantify changes in ruminal bacterial populations, and 2) determine *in vitro* capacity for growth on lactate medium following intraruminal dosing with *Megasphaera elsdenii* strain NCIMB 41125. Treatments consisted of inoculation with a placebo (100 mL of autoclaved culture) or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125 (10, 100, or 1000 mL of fresh culture). Cattle were blocked by initial BW, assigned randomly to treatments, placed into individual pens, and allowed *ad libitum* access to alfalfa hay, salt, and water for a 3-wk adaptation period. Treatments were administered via the ruminal cannula following 24 h of feed and water deprivation. Immediately after dosing, steers were given *ad libitum* access to a diet consisting of 34% roughage and 66% concentrate. Ruminal samples were collected at 0, 2, 4, 6, 8, and 24 h after feeding for quantitative rt-PCR detection of native and introduced strains of *M. elsdenii*, as well as total bacterial genomes. Capacity for growth on lactate medium at each collection time after feeding was evaluated by inoculating 0.2 mL of strained ruminal fluid into anaerobic culture tubes containing 15 mL of semi-defined lactate medium. Tubes were incubated at 39˚C, and turbidity changes were determined by measuring absorbance at 2-h intervals up to 12 h post-inculation. Total number of bacterial genomes 24 h after inoculation was unaffected by intraruminal dosing of *M. elsdenii* (P >0.05). Populations of total (native plus introduced strains) *M. elsdenii* and *M. elsdenii* strain NCIMB 41125 increased by 24 h after inoculation (P <0.05). Turbidity of cultures containing lactate medium increased in response to *M. elsdenii* administration (P <0.05), suggesting a greater capacity for lactate utilization in inoculated cattle compared to the placebo group.

Inoculating cattle with *M. elsdenii* is effective in bolstering populations of ruminal lactate utilizers, and may be useful in preventing ruminal lactate accumulation in cattle during adaptation to high-concentrate diets.

Introduction

Newly arrived feedlot cattle are not normally adapted to high-concentrate diets. The microorganisms inhabiting their rumens reflect populations that are suited to digestion of forages rather than non-structural carbohydrates. Feedlot cattle also experience a great deal of stress during marketing and upon arrival at the feedlot (Hutcheson and Cole, 1986; Loerch and Fluharty, 1999). Transportation to a feedlot can be stressful for many reasons (Grandin, 1997), and can have substantial impact on feed intake following arrival (Brown et al., 2006). The combined effects of stress from transportation and deprivation of feed and water also may disrupt ruminal microbial populations (Galyean et al., 1981). Exposure of feedlot cattle to diets rich in concentrates is known to cause marked changes in the ruminal environment, and the ruminal microflora normally require a period of time to become stable (Bevans et al., 2005). In cattle unaccustomed to eating readily-fermented carbohydrates, higher levels of lactic acid in the rumen may cause mild to severe acidosis. Adaptation to concentrates typically takes from two to four weeks, during which time digestive disturbances such as acidosis, diarrhea and bloat may occur. These conditions may result in poor performance, increased morbidity rates, and greater mortality.

The rumen of cattle is one of the most extensively studied microbial ecosystems (Piknova et al., 2006). Counotte et al. (1981) reported that *M. elsdenii* is the most important organism contributing to the utilization of lactate in the rumen of cattle. According to Russell et al. (1981), *M. elsdenii* has a key role in the prevention of ruminal acidosis. For the past 50 years, cattle

feeders have exploited the use of cereal grains in diets fed to beef cattle to optimize performance (Russell and Rychlik, 2001). The beef industry is well known for feeding high-carbohydrate rations that can give rise to increased lactic acid production within the rumen (Hungate et al.; 1952; Bauman and Foster, 1956). Bolstering the population of lactate utilizing bacteria (**LUB**) within the rumen could prove very useful in controlling acidosis.

It is hypothesized that dosing newly arrived cattle with *M. elsdenii*, a key LUB that is commensal to the rumens of grain-adapted cattle, will facilitate the transition from roughage-based diets to diets that contain high concentrations of non-structural carbohydrates. *M. elsdenii* is a key LUB in feedlot cattle being fed high-grain diets. As described by Elsden and Lewis (1953), *M. elsdenii* are Gram-negative, non-motile, large cocci that produce fatty acids and are strictly anaerobic. When feedlot cattle are rapidly shifted from diets consisting primarily of forage to diets that contain large amounts of readily fermented carbohydrates, lactate production is greater than utilization because amylolytic bacteria proliferate at a rate faster than LUB species (Mackie and Gilchrist, 1979). Counotte et al. (1981) found that *M. elsdenii* seems to be the predominant LUB within the rumen, fermenting up to 97% of ruminal lactate. *M. elsdenii* could be useful as a probiotic if inoculated into un-adapted feedlot cattle prior to exposing them to high-grain diets (Kung and Hession, 1995). In fact, Robinson et al. (1992) reported that steers dosed intraruminally with *M. elsdenii* had reduced lactate concentrations, higher ruminal pH, and 24% greater DMI when compared to control animals. In another experiment conducted by Hibbard et al. (1993), oral drenching with *M. elsdenii* prevented lactic acidosis and improved DMI of cattle switched abruptly from a 50% to a 90% concentrate diet.

Scientists at the Agricultural Research Center in Irene, South Africa, isolated a prolific bovine strain of *Megasphaera elsdenii*, herein referred to as *M. elsdenii* NCIMB 41125. This

strain of *M. elsdenii* was the subject of our investigation. Our objectives were to 1) determine efficacy of increasing ruminal populations of LUB in cattle with oral dosing of *M. elsdenii* NCIMB 41125; 2) determine efficacy for preventing ruminal accumulation of lactic acid in cattle with oral dosing of *M. elsdenii* NCIMB 41125; 3) compare dosages consisting of 1.62×10^{9}, 1.62×10^{10}, and 1.62×10^{11} cfu of *M. elsdenii* NCIMB 41125, and 4) assess persistence of the introduced strain.

Materials and Methods

All procedures followed in the present study were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol no. 2535).

Crossbred Angus steers (n= 20; initial BW=253 ±24 kg) were purchased from commercial sale barns in Salina and Manhattan, KS, and transported to the Kansas State University Beef Cattle Research Center. Upon arrival, steers were offered ad libitum access to alfalfa hay and water prior to processing. At initial processing steers were weighed, vaccinated for viral and clostridial diseases (Bovi-Shield 4 and Ultrabac 7, Pfizer Inc.; Exton, PA), given an external parasiticide (Phoenectin; VX Animal Health., St. Joseph, MO), and identified with uniquely numbered ear tags. Steers were fitted with ruminal cannulas (Bar Diamond, Parma, ID), and housed individually in slatted floor pens measuring 1.5 m × 3 m each. Pens were equipped with individual feed bunks and automatic water fountains that allowed *ad libitum* access to alfalfa hay, salt, and clean water.

Following a 3-wk recovery period, cattle were blocked by BW and assigned randomly, within blocks, to one of four treatments. Treatments consisted of intraruminal dosing with a placebo (100 mL of autoclaved culture), or 10, 100, or 1,000 mL of a live culture containing 1.62×10^{8} colony forming units of *Megasphaera elsdenii* NCIMB 41125. *M. elsdenii* was grown

in continuous culture under anaerobic conditions at 39˚C and at pH 5.5 using a Sartorius BIOSTAT® C-DCU (Sartorius BBI Systems, Melsungen, Germany) bioreactor with a dilution rate of 40%. A semi-defined lactate medium (Table 3-2) was used to support growth of the organism, and nitrogen gas was used to maintain anaerobic conditions. Bacterial cultures were harvested anaerobically into stainless steel kegs.

To allow for comparable post-feeding sampling times for all animals, the first animal in the row of 20 pens was fed at 0800 h, and successive animals were fed at 5-min intervals thereafter. This provided sufficient time for feeding, inoculating, and sampling, thus making it possible to retrieve ruminal digesta at predetermined intervals after feeding. The same feeding and sampling sequence was followed throughout the trial (Figure 3-1). On day 1, background samples were taken immediately prior to feeding. On days 2 through 7, samples of ruminal digesta were taken at 0, 2, 4, 6, 8 and 24 h post-feeding. On days 12, and 17, samples were taken at 0, 2, 4, 6 and 8 hours post-feeding. On days 24, 31 and 42, samples were taken only at 4 h post-feeding. At each sampling point, the animal was haltered and tied to the side panel of the stall, the ruminal fistula cap was removed, and four handfuls of contents were removed from the cranial and ventral sacs of the rumen and placed into a plastic container. The contents of the container were strained through four layers of cheesecloth, and the strained rumen fluid was placed into scintillation vials and frozen for subsequent analysis.

At 0800 h on day 1 of the experimental period, background samples were taken from animals to establish ruminal conditions prior to exposing animals to readily fermented carbohydrates. Steers were fasted for 24 h, and beginning at 0800 h on day 2, ruminal digesta was collected from each animal (hour 0), and the appropriate inoculum was then administered as a liquid suspension via the rumen cannula. Immediately after dosing and sampling, steers were

fed a 66% concentrate-based diet for the first time. Steers were given *ad libitum* access to a steam-flaked corn-based diet with 66% concentrate and 34% roughage (Table 3-1). On Day 7, cattle were changed to a diet consisting of 80% concentrate, again using steam-flaked as the principal energy source. On day 12, steers were changed to the final diet consisting of 94% concentrate.

Approximately 15 mL samples of freshly strained ruminal fluid were collected at each time point and placed into duplicate scintillation vials for subsequent determination, by quantitative rt-PCR and cell flow cytometry, of total bacterial genomes, undifferentiated *M. elsdenii* genomes, and *M. elsdenii* strain NCIMB 41125. Scintillation vials were placed on ice for approximately 90 min, and then stored frozen at -20˚C. Samples were packaged into boxes and shipped on dry ice to Alimetrics, Ltd. (Helsinki, Finland) to be analyzed for ruminal populations of *M. elsdenii* (species and strain) and total bacterial numbers, using quantitative rt-PCR and cell flow cytometry. Alimetrics, Ltd. developed a PCR primer with a detection limit of 2.6×10^4 cfu/mL based on 16S rRNA for the strain-specific analysis of *M. elsdenii* used in our research.

During the *in vitro* portion of the experiment, culture tubes containing 15 mL of a semi-defined lactate medium (Table 3-2) were inoculated with 0.2 mL of strained ruminal fluid. Injection surfaces of culture tubes were flame-sterilized and inoculated using sterile 1 mL tuberculin type slip-tip syringes and 20 g needles. Immediately following inoculation, tubes were vortexed and absorbance at 600 nm was measured using a Spectronic 20D+ (Thermo Fisher Scientific, Inc., Waltham, MA) spectrophotometer. Following measurement of initial optical density, the tubes were placed into an incubator at 39˚C. Optical density was measured after vortexing every two h post-inoculation up to 12 h.

Statistical Analysis

Data for total ruminal bacterial populations and *M. elsdenii* populations (species and strain-specific) were analyzed as repeated measures using the MIXED procedure of SAS. The model statement included the effects of treatment, sampling time, and the interaction. Weight block served as the random effect.

Data for the *in vitro* analyses were analyzed as repeated measures using the MIXED procedure of SAS. The model statement included the effects of treatment, sampling time, and period. Weight block served as the random effect.

Contrasts for both models included a comparison of the placebo group to the average of all groups administered live *Megasphaera elsdenii* NCIMB 41125, as well and the linear and quadratic effects of dosage size

Results and Discussion

Ruminal Microbial Populations

Pre-challenge bacterial populations within the rumens of steers were similar among treatment groups (Table 3-3). Populations of *M. elsdenii* prior to inoculation and the carbohydrate challenge are shown in Table 3-2. As expected, initial populations of *M. elsdenii* in forage-fed cattle were very low. Introduction of highly fermentable substrates, such as steam-flaked corn, is conducive to production of lactate by *Streptococcus bovis* and other lactate-producing species. In the absence of significant quantities of lactate, populations of *M. elsdenii*, one of the key lactate-utilizing species, typically will be quite low. Twenty four h after inoculation, total bacterial genomes were approximately 10^{10} cfu/mL and were unaffected by intraruminal dosing of *M. elsdenii* strain NCIMB 41125 ($P > 0.05$). Total bacterial genomes remained at this level throughout the study. Populations of undifferentiated *M. elsdenii* increased

($P < 0.05$) by 24-h after inoculation (Figure 3-2). A linear effect ($P < 0.05$) of dose volume on undifferentiated ruminal *M. elsdenii* populations was observed, which was consistent with increases in populations of *M. elsdenii* strain NCIMB 41125 ($P < 0.05$; Figure 3-3). In the strain specific analysis, a linear effect of dose volume was observed ($P < 0.05$). On d 3, undifferentiated *M. elsdenii* populations in the placebo group increased gradually, but not at the same rate as steers dosed with live cultures ($P < 0.05$). Day 3 undifferentiated populations of *M. elsdenii* are shown in Figure 3-2. In the strain specific analysis for ruminal populations of *M. elsdenii* NCIMB 41125, steers dosed with live cultures had increased populations on d 3 ($P < 0.05$; Figure 3-3). By d 5, ruminal populations of *M. elsdenii* in the placebo group had increased in comparison to the previous sampling, but were lower than those of steers dosed with live cultures ($P < 0.05$; Figure 3-2).

Compared to the placebo group, populations of the introduced strain remained greater for d 5 and d 7 for steers dosed with live cultures ($P < 0.05$; Figure 3-3). Following the change to an 80% concentrate diet on d 7, populations of *M. elsdenii* remainedly higher for steers dosed with the live culture versus the placebo group ($P < 0.05$).

On d 12, diets were stepped up to 94% concentrate. Since the cattle had been on a steam-flaked corn-based diet for 10 d, it is logical that the population of *M. elsdenii* in the placebo group would increase. Ruminal *M. elsdenii* populations were not different among treatments beyond this time.

It is important to note the detection limit of 2.6×10^4 cfu/mL in the analysis. Background levels of the strain specific organisms being present in the placebo group can be explained by the probe reacting with other 16S rRNA genes possessing similar DNA sequences as the introduced organism. Our observations are supportive of the hypothesis that dosing cattle with an introduced

strain of *M. elsdenii* can be functional in increasing ruminal populations of lactate-utilizing bacteria.

In vitro Growth on Lactate Medium

Prior to introduction of the challenge diet and inoculation, there were no differences among treatments with respect to *in vitro* capacity for utilization of lactic acid (Figure 3-4). On d 2, 2 h post-inoculation (and following introduction of the 66% concentrate diet), we observed differences among treatments in terms of capacity for lactate utilization. Steers dosed with live *M. elsdenii* yielded ruminal fluid with greater capacity for growth on lactate medium compared to the placebo group ($P < 0.05$), as well as linear and quadratic effects of dose size ($P < 0.05$). During the post-inoculation period, the 1000 mL dose revealed more *in vitro* growth; (Figure 3-4). As shown by Figure 3-4, four hours post-challenge and inoculation, we found the same scenario, with treatment by time (time post-incubation) interactions ($P < 0.05$), and linear effects of dosage size as compared to the placebo ($P < 0.05$). In our analysis of 6 and 8 h post-inoculation, we observed treatment by time (time post-incubation) interactions ($P < 0.05$), and linear effects of dosage size among the steers dosed with live *M. elsdenii* as compared to the placebo ($P < 0.05$; Figure 3-4). On d 3, (24 through 32 h) post-inoculation and introduction to a highly fermentable diet, we observed the same scenario with treatment by time interactions ($P < 0.05$), and linear effects of dosage size and differences among the steers dosed with live *M. elsdenii* as compared to the placebo ($P < 0.05$; Figure 3-4). By 72 h, D 5, we found no differences among treatment groups relative to the *in vitro* growth on lactate medium.

This portion of our study provides important information pertaining to *M. elsdenii* and its ability to utilize lactate and proliferate at significantly different rates as compared to a placebo. The *in vitro* growth in lactate medium by cultures obtained from steers administered live doses

provides information that supports our hypothesis that inoculating cattle with *M. elsdenii* constitutes a viable means of establishing significant populations of an important LUB within the rumens of cattle prior to feeding a highly fermentable diet.

Assessing Dose Size

A key objective of this experiment was to asses the effects of dose size. As dose size increased, the population bacteria in the inoculum also increased. On the day of inoculation, we observed a linear effect of dose size in ruminal populations of undifferentiated *M. elsdenii* as well as the introduced strain ($P < 0.05$). By 24-h post-inoculation (the morning of d 3), no differences were noted among ruminal populations of *M. elsdenii* ($P < 0.05$). Ruminal populations of NCIMB 41125 grew to the same level, regardless of dose size.

Naturally occurring, live bacterial supplements (probiotics or direct-fed microbials) have the potential to decrease the incidence of acidosis in feedlot cattle being adapted to rapidly fermentable diets (Ghorbani et al., 2002). Using *in vitro* and *in vivo* studies, inoculating cattle with *M. elsdenii* has been shown to modify ruminal fermentation and reduce the accumulation of lactic acid during diet changes experienced by newly-received feedlot cattle (Greening et al., 1991; Kung and Hession, 1995). When considering the use of *M. elsdenii* as an intervention, it is paramount to consider the abilities of the organism to proliferate and establish a viable ruminal population (Ouwerkerk et al., 2002). Our results verify that dosing cattle with *M. elsdenii* strain NCIMB 41125 can successfully amplify existing ruminal populations of autochthonous *M. elsdenii*, as well as increase populations of the introduced strain.

Conclusion

If producers in the United States could alter the ruminal microflora of newly arrived feedlot cattle to increase LUB, accumulation of detrimental levels of lactate in the rumen could

very well be avoided. *In vivo* studies involving the NCIMB 41125 strain of *M. elsdenii* have demonstrated the ability to bolster populations of this key lactate-utilizing bacteria in the rumen. *In vitro* studies have proven that organisms in rumen fluid from cattle dosed with *M. elsdenii* has a greater capacity for growth on lactate medium.

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Table 3-1. Composition of experiment diets of steers.

	Concentrate, % of DM		
Ingredient, % of DM	66%	80%	94%
Steam-flaked corn	56.9	70.8	84.4
Ground alfalfa hay	33.2	19.4	5.8
Corn steep liquor	6.5	6.5	6.4
Vitamin-mineral premix[1]	3.4	3.4	3.3
Nutrient Composition			
CP, %	15.7	14.8	14.0
NE_m, Mcal/kg	1.87	2.05	2.20
NE_g, Mcal/kg	1.23	1.39	1.54
Ca, %	1.05	0.88	0.71
P, %	0.36	0.37	0.38

[1]Formulated to provide 0.1 mg/kg Co, 10 mg/kg Cu, 0.6 mg/kg I, 60 mg/kg Mn, 0.25 mg/kg Se, 60 mg/kg Zn,
0.88 % K, and 2205 IU/kg of vitamin A.

Table 3-2. Semi-defined lactate medium.

Ingredient	Amount, g/L
Distilled H_2O	960
Sodium Lactate (60% w/v)	16.67
Indigocarmine (0.5%)	1
Mineral solution	25
Peptone	3
Yeast extract	3
Vitamin solution	2
12.5% L-cysteine	2
12.5% Sodium Sulfide	2

Table 3-3. Ruminal bacterial populations of cattle prior to being dosed with a placebo or live cultures of *Megasphaera elsdenii* strain NCIMB 41125.

	CFU of M. elsdenii strain NCIMB 41125				
Product[a]	0	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM
Total Genomes, genomes/mL	10.1	9.8	9.7	9.8	0.21
Undifferentiated *M. elsdenii*, cfu/mL	4.7	4.5	4.5	4.9	0.44
M. elsdenii strain NCIMB 41125[b]	4.6	4.4	4.5	4.9	0.44

[a]All counts are in genomes/mL, $\log_{10}$.
[b]Detection limit of assay was 2.6×10^{4} genomes/mL.

Figure 3-1. Timeline of sampling times and dietary changes.

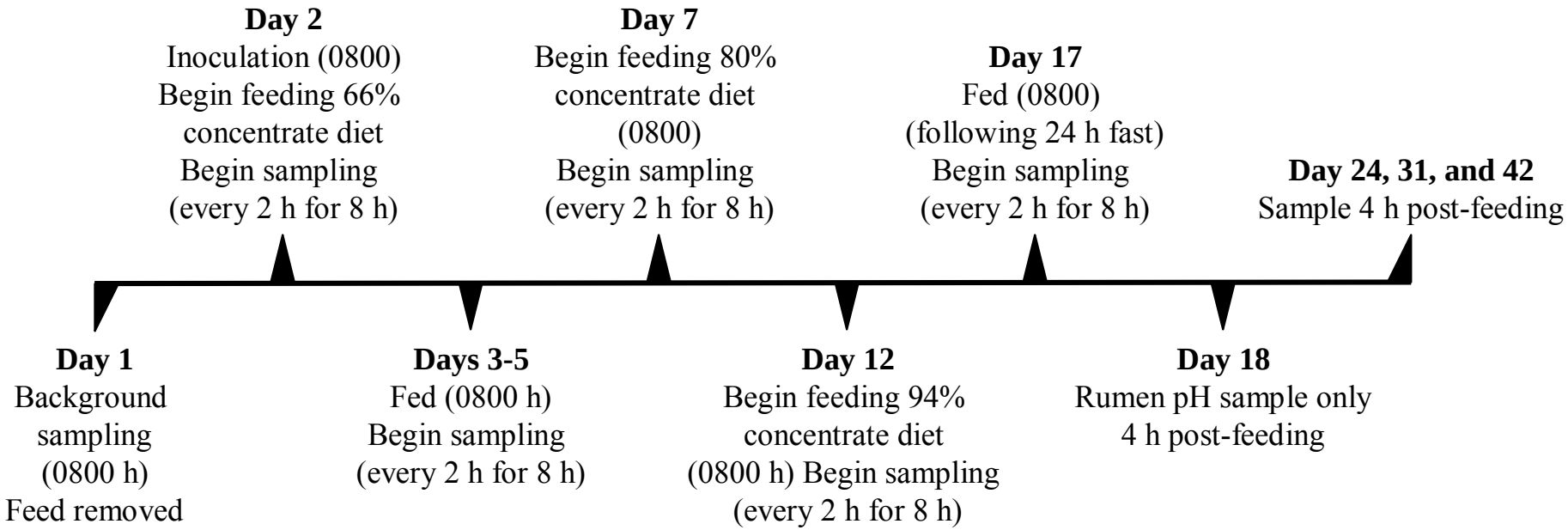
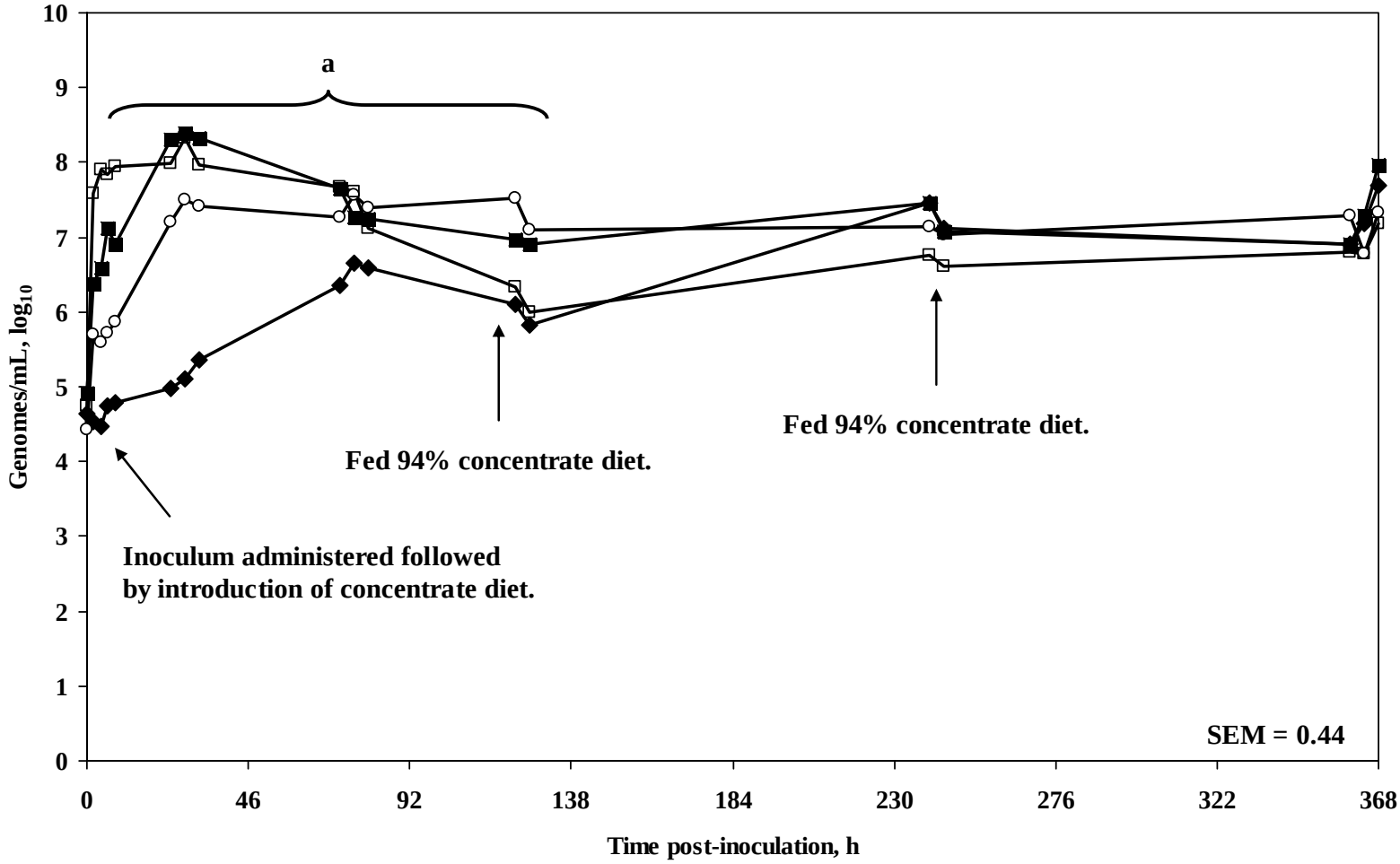
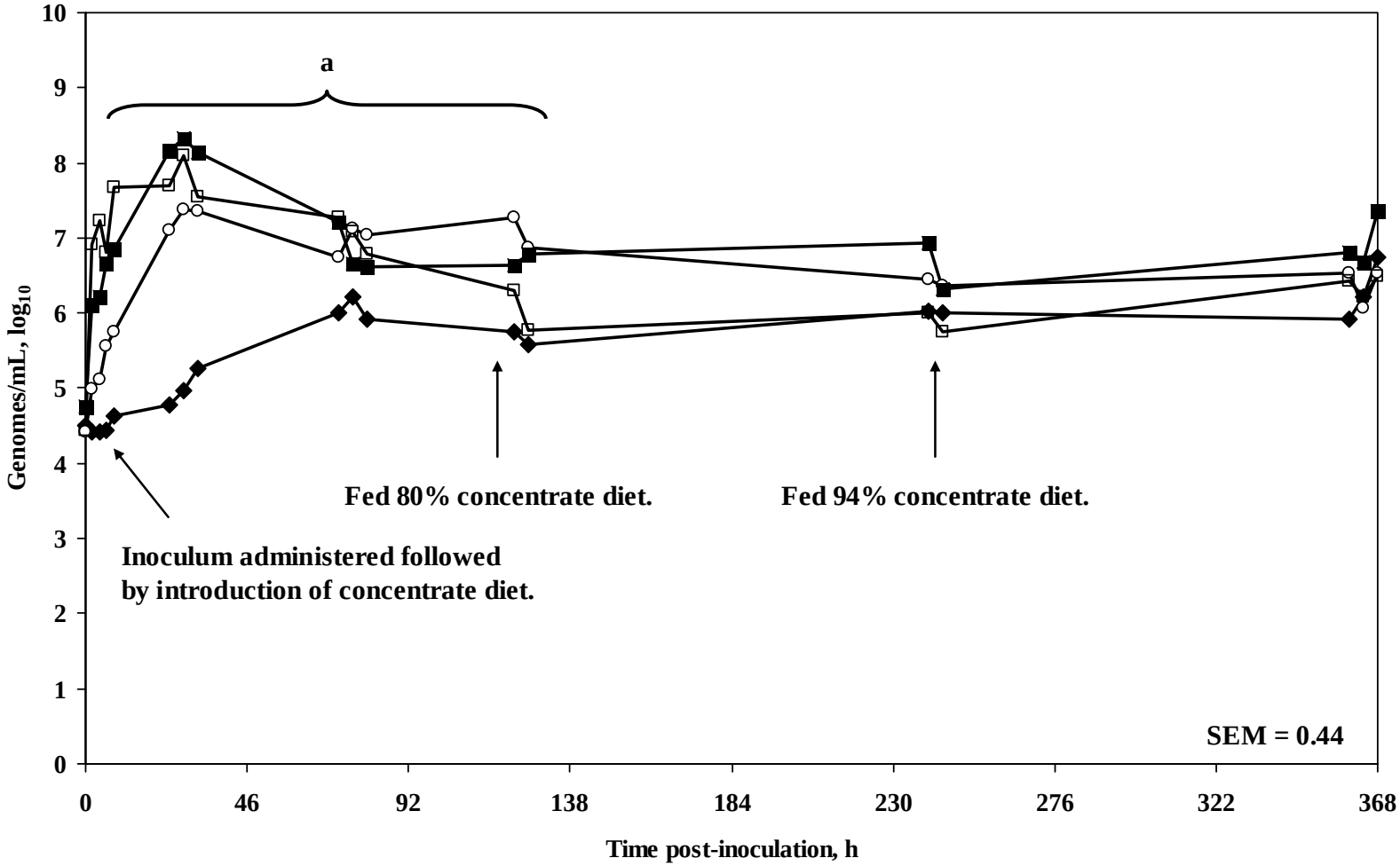


Figure 3-2. Day 2 through 17 ruminal populations of undifferentiated *M. elsdenii* (day of challenge) in cattle dosed with a placebo or live cultures of *Megasphaera elsdenii* strain NCIMB 41125.
(Placebo —□—, 10 mL —○—, 100 mL —■—, 1000 mL —◆—)



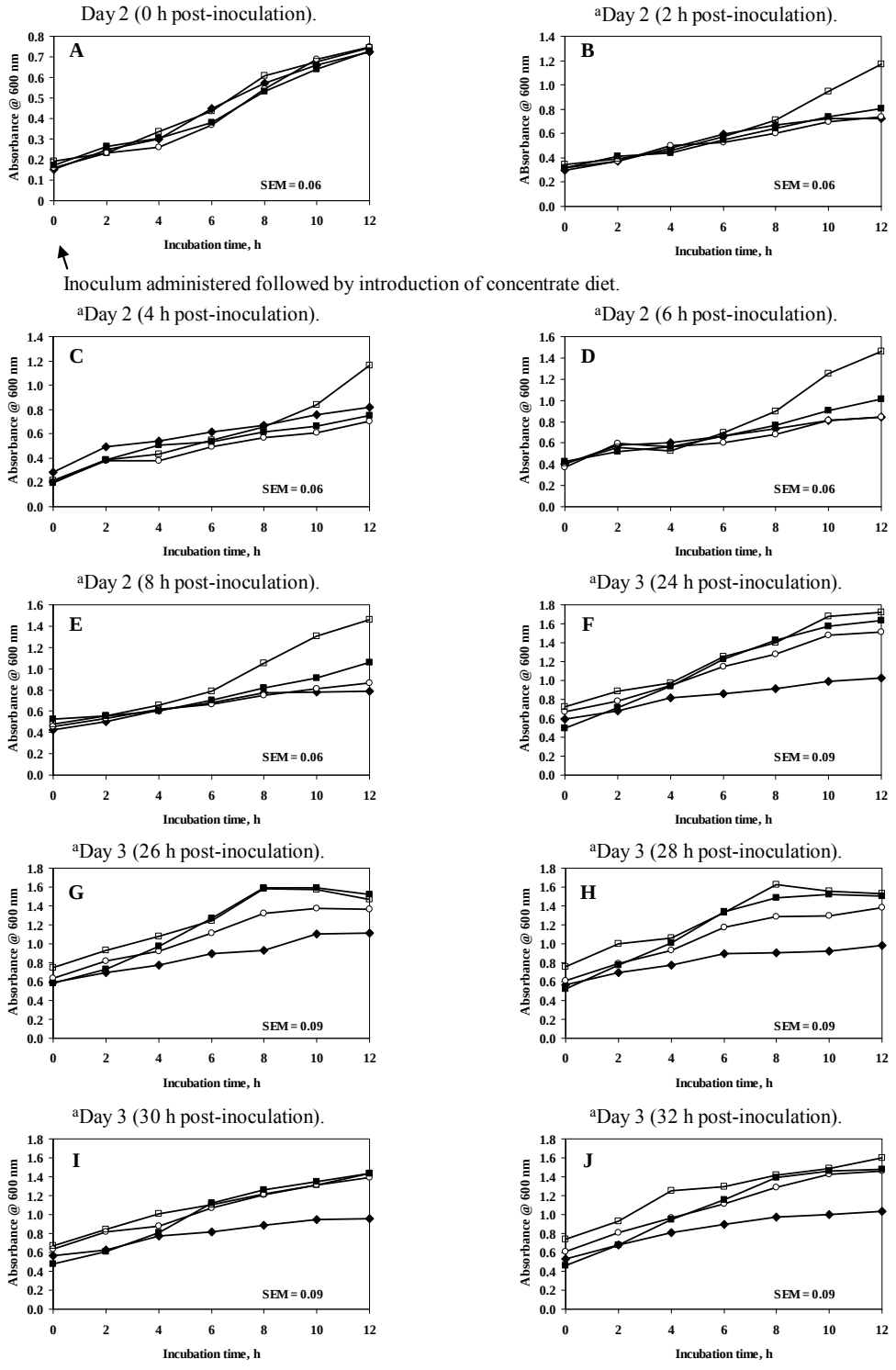
[a]**Treatment effect ($P < 0.05$); Treatment × time Interaction ($P < 0.05$); Linear effect of dosage size ($P < 0.05$).**

Figure 3-3. Day 2 through 17 total ruminal *M. elsdenii* strain NCIMB 41125 populations (day of challenge) of cattle dosed with a Placebo or live cultures of *Megasphaera elsdenii* strain NCIMB 41125.
(Placebo —◆—, 10 mL —○—, 100 mL—■—, 1000 mL —□—)



[a]**Treatment effect ($P < 0.05$); Treatment × time Interaction ($P < 0.05$); Linear effect of dosage size ($P < 0.05$).**

Figure 3-4 (A through J). *In vitro* growth on lactate medium.
(Placebo —◆—, 10 mL —○—, 100 mL —■—, 1000 mL —□—)



[a]Treatment x incubation time interaction ($P < 0.05$); Linear effect of dose size ($P < 0.05$); Placebo vs *Megasphaera* groups ($P < 0.05$).

CHAPTER 4 - Oral dosing of *Megasphaera elsdenii* strain NCIMB 41125 to feedlot cattle at initial processing: effects on subsequent feedlot performance, health, and carcass characteristics of cattle produced under a natural feeding regimen

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Abstract

A study was conducted in a commercial feedlot to evaluate the efficacy of *Megasphaera elsdenii* strain NCIMB 41125 when administered to feedlot cattle. A second objective of the study was to determine if oral dosing of *M. elsdenii* could decrease the number of cattle treated for bovine respiratory disease, recognizing that symptoms of BRD are very similar to those associated with acidosis, and that the occurrence of acidosis can also predispose cattle to respiratory disease. All cattle were fed under a strict natural beef program endorsed by Certified Angus Beef® Natural brand (**CAN**). Cattle being fed under this regimen are not implanted, must be fed all natural diets containing no animal by-products, and without antibiotic or steroidal drug use. Angus steers and heifers (n = 3179; average BW = 356 ± 58.4 kg) were used in a randomized complete block design with two treatments. Cattle were assigned to treatment on an every-other-head basis at initial processing. Cattle in the treatment group were orally drenched with 100 mL of a culture medium containing 1.5×10^8 cfu/mL of *M. elsdenii* strain NCIMB 41125 using a pneumatic drenching gun. Lot tags were color coded to facilitate identification of each treatment group. Cattle were blocked by gender (steers and heifers) and by date of arrival, with a total of ten replications (20 pens). Following initial processing, cattle were assigned to a home pen for the remainder of the finishing period and were not mixed with cattle from different pens at any time. Total weight of each pen was determined upon initiation of the experiment and immediately before shipping to a commercial abattoir. Data obtained for each pen of cattle included initial weight, DMI, finishing weight, carcass weight, dressing percentage, USDA quality grade, USDA yield grade and grid-based CAN program carcass qualifications. Morbidity and mortality data also were collected for each pen. Average daily gain and feed efficiencies were calculated from the pertinent data. Cattle were sorted via visual appraisal, and shipped to a commercial abattoir. Final statistical analyses of the study revealed no differences in feed

efficiency. All carcass calculations were based on a common dressing percentage of 63.5% and a 4% transit shrink. The control group had more USDA yield grade 2 carcasses ($P < 0.05$), and cattle dosed with *M. elsdenii* had more USDA yield grade 5 carcasses ($P < 0.05$). Cattle in the dosed group also tended to yield a higher percentage of USDA Prime carcasses ($P = 0.14$). No effects were observed in comparison of liver abscesses among either group. *M. elsdenii* is known to be an important lactate utilizing bacteria (**LUB**) in cattle being fed highly fermentable carbohydrate rations. Though no differences were observed in the current study concerning feedlot performance, it is important to consider the amount of roughage fed in a natural production regimen. Inclusion of more roughage in the diets of feedlot cattle may also reduce lactate levels and incidences of acidosis, which may have shadowed the effects of dosing cattle with a ruminal bacteria that has been proven to be successful in the removal of lactic acid. Dosing cattle with *M. elsdenii* may also have effects on economically important parameters such as yield and quality grades. Dosing did not have an effect on BRD.

Introduction

Acidosis in feedlot cattle is among the most frequent problems associated with feeding high-grain rations. Ruminal acidosis afflicts large numbers of cattle, and is associated with decreased productivity, as well as increased incidence of important maladies such as bloat, founder, and liver abscesses. Acidosis has been well known since the inception of grain utilization in the cattle-feeding industry (Nagaraja and Lechtenberg, 2007). High energy diets with 80 to 95% concentrate can readily lead to the development of ruminal acidosis (Krehbiel et al., 1995). Grain-rich diets are highly fermentable and contain large amounts of starch, sucrose, lactose, or glucose (Hungate et al., 1952). Predisposition to acidosis is greatest when cattle are

transitioned from high roughage diets to grain diets. Cattle normally are transitioned to high-grain diets over a period of 2 to 3 wk, and changes in diet composition frequently result in development of acidosis (Elam, 1976). The microbial population within the rumen is diverse, and its composition is largely a function of the types of energy substrates fed. When the diet is changed from slow digesting fibrous feeds to rapidly digesting starch-based feeds, opportunistic bacteria quickly populate the rumen. Increases in Gram-positive, lactic-acid producing bacteria, destruction of the Gram-negative bacteria, and a reduction or complete defaunation of ciliated protozoa may occur (Nagaraja et al., 1998). Gram-positive organisms rapidly digest starches and form end products (organic acids) that can have deleterious consequences if not removed from the rumen. Removal of excess acids is the task of other types of bacteria, including *M. elsdenii,* but they generally are slower to populate the rumen, responding to supply of lactic acid as substrate.

Using *in vitro* and *in vivo* studies, inoculating cattle with *M. elsdenii* has been shown to modify ruminal fermentation and prevent accumulation of lactic acid caused by the ruminal fermentation of grain (Greening et al., 1991; Kung and Hession, 1995). Consequently, placing large numbers of *M. elsdenii* into the rumen prior to introduction of grains may make it feasible to avoid or reduce the magnitude of acidosis during the step-up period, thereby maintaining higher feed intakes and improved productivity. Robinson et al. (1992) dosed steers intraruminally with *M. elsdenii* and reported reduced lactate concentrations, higher ruminal pH, and 24% greater DMI when compared to control animals. Another experiment by Hibbard et al. (1993) found that oral drenching with *M. elsdenii* prevented lactic acidosis and improved intake of cattle switched from a 50% to 90% concentrate diet. Inoculation with *M. elsdenii* holds promise for the prevention of the accumulation of lactate in the rumen of unadapted feedlot cattle

as well as shortening the time for adaptation to high-grain diets (Kung and Hession, 1995). *M. elsdenii* is a very important LUB that is commonly found within the ruminal microflora of cattle being fed concentrate-based diets. *M. elsdenii* is slower to populate the rumen than other organisms, such as *Streptococcus bovis,* after the introduction of common highly-fermentable non-structural carbohydrates into the diets of feedlot cattle. Dosing feedlot cattle with a strain of *M. elsdenii* such as NCIMB 41125 that is known to be very prolific in the presence of high-grain diets could be an effective preventative measure.

Natural Beef Production

The use of ionophores and other antiobiotic feed additives have allowed producers to be more profitable. Growth in demand for beef produced without the aid of these products has fueled interests in alternative strategies for acidosis prevention and control.

Naturally produced beef is becoming more popular (Boland and Schroeder, 2002). Finishing beef cattle without the use of implants, antibiotics, and ionophores has disadvantages, predisposing animals to metabolic disorders such as acidosis. Although the use of drugs is not allowed in some programs for "natural" beef, use of direct-fed microbials generally is permitted. *Megasphaera elsdenii*, a lactate utilizer, could therefore prove useful as a means of preventing disorders in feedlot cattle.

Scientists at the Agricultural Research Council in Irene, South Africa, isolated a prolific bovine strain of *Megasphaera elsdenii*, herein referred to as *M. elsdenii* NCIMB 41125. This strain of *M. elsdenii* was the subject of our investigation. Our objectives were to evaluate the efficacy of *M. elsdenii* strain NCIMB 41125 when administered to feedlot cattle produced under a natural feeding regimen, and to determine if administration of *M. elsdenii* has potential to decrease the number of cattle treated for bovine respiratory disease.

Materials and Methods

Cultures of *M. elsdenii* strain NCIMB 41125 were grown in the laboratory at Kansas State University for use in this study. They were grown in continuous culture under anaerobic conditions at 39˚C and pH maintained at 5.5 using a Sartorius BIOSTAT® C-DCU (Sartorius BBI Systems, Melsungen, Germany) bioreactor with a dilution rate of 40%. A semi-defined lactate medium was used to support growth of the inoculum. Nitrogen gas was used to maintain anaerobic conditions. Bacterial cultures were harvested anaerobically into stainless steel kegs. Bacterial counts on each day of dosing are given in Table 4-1. Angus and Angus crossbred steers and heifers (n = 3179; average initial BW = 356 ± 58 kg) were used in a randomized complete block design with two treatments. At in initial processing cattle were weighed, vaccinated for viral (Titanium® IBR, AgriLabs; St. Joseph, MO) and clostridial (Vision 7®, Intervet, Inc.; Millsboro, DE) diseases, given an injectable parasiticide (Ivomec® Plus, Merial; Duluth, GA), assigned to treatment (every-other-head), and tagged with color coded tags. Cattle that were orally dosed with *M. elsdenii* strain NCIMB 41125 received 100 mL of fresh culture. Cattle assigned to the control group did not receive an oral drench. Following initial processing and treatment administration, cattle were placed in home pens for the duration of the finishing period. A total of 20 pens, with 10 blocks per treatment were used. Cattle were not mixed with other pens any time during the trial period. The feedlot facilities used were typical of a commercial feeding operation. Pens were equipped with automatic water fountains and fence-line bunks. Feeding regimens consisted of a slick-bunk feed-management system in which cattle were fed 3 times daily. Experimental diets are given in Table 4-2. Normal feedlot standard operating procedures were performed daily. Cattle that required medical attention were treated promptly. Treatment requiring antibiotics or other drugs not permissible in the CAN program resulted in

subsequent removal from study. In the event of mortality, a post-mortem diagnosis was performed on the animal.

Visual appraisal was used to determine when individual animals in a pen were ready for harvest. Multiple points of harvest were necessary to allow all cattle to achieve optimum marketability. Cattle identified as being ready for harvest were sorted into a holding pen. Cattle were weighed using a pen scale and shipped to a commercial abattoir in Lexington, NE. A 4% transit shrink was used in calculation of live weights upon arrival to the abbatoir. For carcass calculations, a common dressing percentage of 63.5% and a 4% transit shrink were used. Liver abscess scores were taken using the Elanco system where: A- = 1 or 2 small abscesses or abscess scars; A^0 = 2 to 4 well organized abscesses; or A+ = 1 or more large active abscesses with inflammation or adhesion to surrounding tissue (Depenbusch et al., 2008). Hot carcass weights were recorded and USDA yield grades and quality grades were assigned by USDA graders.

Statistical Analysis

The statistical design of the current study was a randomized complete block with two treatments. Model effects included treatment (with and without *M. elsdenii*) and total number of animals. Feedlot pen constituted the experimental unit and block was used as the random effect. Treatment differences were evaluated using the MIXED models procedure of SAS 9.1 (SAS Inst. Inc., Cary, NC). Cases of mortality and morbidity were calculated as percent of occurrences.

Results and Discussion

Growth, Performance and Carcass Data

Final analyses of the current experiment resulted in no differences ($P > 0.05$) among treatments for growth performance (Table 4-3). Minor differences were observed among treatments with respect to carcass characteristics (Table 4-4 and 4-5). In the control group, we

observed more USDA yield grade 2 carcasses ($P < 0.05$). Cattle dosed with *M. elsdenii* had more USDA yield grade 5 carcasses ($P < 0.05$) and tended to produce more USDA Prime carcasses ($P = 0.13$). In our analyses of liver abscesses, no significant effects of treatment were observed. Overall, the effects of dosing cattle with *Megasphaera elsdenii* were small.

Animal Health

Animal health is an integral part of feedlot management regimens. In value-added programs such as CAN beef, health issues and subsequent treatments directly impact acceptability, as cattle cannot remain in the program after receiving therapeutic drugs. Prevention and control of diseases in natural cattle is therefore paramount. Feedlot cattle experience a great deal of stress during marketing and upon arrival at the feedlot (Hutcheson and Cole, 1986; Loerch and Fluharty, 1999), which can lead to greater incidences of disease. Additionally, clinical manifestations of BRD are difficult to distinguish from those of acidosis, potentially leading to misdiagnosis and inappropriate therapeutic treatment. Prevention of acidosis may, therefore decrease the incidence of both real and perceived cases of BRD.

In our analysis of morbidity in the current study, there were three health issues noted. Cattle were afflicted with respiratory disease, acute interstitial pneumonia, or diphtheria. Table 4-6 summarizes occurrences of morbidity, identifying the percentages of animals removed from study for various reasons. We found no statistical differences among study treatments for any of the disorders. Table 4-7 summarizes the diagnosed causes of mortality. Animal losses were due to heart failure, bloat, pneumonia, acute interstitial pneumonia, or were euthanized as a result of injuries. Based on morbidity and mortality records, no significant differences were found among the treatment groups for any of the causative factors.

Conclusion

Minor effects of dosing cattle with *M. elsdenii* were observed in carcass characteristics. Dosing did not have any effects on subsequent feedlot performance. No differences were noted among treatments concerning morbidity or mortality. Under the conditions used in the experiment, dosing cattle with *M. elsdenii* strain NCIMB 41125 had no significant effects on health or performance. Perhaps, an insufficient challenge offset the results of dosing.

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Table 4-1. Counts of *M. elsdenii* in kegs by starting date.

Starting Date	Count (cfu/mL)
6-5-2007	9.02^{7}
6-15-2007	1.11^{8}
6-23-2007	3.16^{8}
9-13-2007	1.04^{8}
9-26-2007	1.42^{8}
10-1-2007	1.31^{8}

Table 4-2. Experimental diets and nutrient composition (formulated) for feedlot cattle assigned to one of two treatments (Control or *Megasphaera*[1]) at initial processing.

	Diets		
Ingredient, (%DM basis)	Starter Ration	Intermediate	Finishing
Steam-flaked corn	34.5	57.5	70.5
Alfalfa hay	43.4	20.8	10.7
Wet corn distillers grains	15.0	13.3	13.3
Corn silage	4.0	4.0	0
Vitamin-mineral pre-mix[2]	3.1	4.4	5.5
Nutrient Composition			
CP, %	16.8	15.0	14.7
NE_m, Mcal,/kg	1.76	1.98	2.04
NE_g, Mcal/kg	1.10	1.28	1.34
Ca, %	1.00	0.75	0.69
P, %	0.31	0.32	0.33
K, %	1.55	1.01	0.75
Ether extract, %	4.0	4.1	4.3

[1]*Megasphaera* = oral drench consisting of 100 ml of a solution containing 1.5×10^8 cfu/mL *M. elsdenii* strain NCIMB 41125; Control = no oral drench.
[2]Formulated to provide 0.4 mg/kg of Co; 20 mg/kg of Cu; 0.5 mg/kg of I; 50 mg/kg of Mn; 0.3 mg/kg of Se; 150 mg/kg of Zn; 40,000 IU/day of vitamin A; and 500 IU/day.

Table 4-3. Growth performance of feedlot cattle assigned to one of two treatments (Control or *Megasphaera*[1]) at initial processing.

Item	Control	*Megasphaera*	SEM	*P*-value
No. of pens (No. animals)	10(1544)	10(1530)	12.9	0.12
Days on feed	153	153	5.2	0.63
Initial BW, kg	355.6	357.4	13.09	0.32
Final BW[2], kg	574.7	575.6	12.29	0.74
DMI, kg/d	10.10	10.03	0.75	0.65
ADG, kg/d	1.44	1.43	0.11	0.28
G:F, g/kg	142.9	142.7	0.3	0.92

[1]*Megasphaera* = oral drench consisting of 100 ml of a solution containing 1.5×10^8 cfu/mL *M. elsdenii* strain NCIMB 41125; Control = no oral drench.
[2]Carcass adjusted final BW calculated by dividing HCW by a common dressing yield of 63.5%.

Table 4-4. Carcass characteristics of feedlot cattle assigned to one of two treatments (Control or *Megasphaera*[1]) at initial processing.

Item	Control	*Megasphaera*	SEM	*P*-value
HCW, kg	364.9	365.5	7.8	0.73
Dress yield, %	63.30	63.31	0.03	0.93
Liver abscess[2], %	42.8	42.2	1.23	0.74
A^+	12.8	12.8	0.87	0.99
A^0	11.4	11.8	0.82	0.72
A^-	18.5	17.7	0.99	0.53

[1]*Megasphaera* = oral drench consisting of 100 ml of a solution containing 1.5×10^8 cfu/mL *M. elsdenii* strain NCIMB 41125; Control = no oral drench.
[2]A^+ = One or more large, or multiple small, active abscesses, with or without adhesions; A^0 = two to 4 small, well-organized abscesses; A^- = one or 2 small abscesses or scars.

Table 4-5. USDA yield and quality grade, and natural carcass merit based program qualifications of feedlot cattle assigned to one of two treatments (Control or *Megasphaera*[1]) at initial processing.

Item	Control	*Megasphaera*	SEM	*P*-value
USDA quality grade, %				
Prime	6.6	7.9	0.64	0.14
Premium Choice	47.5	46.4	1.28	0.54
Choice	38.1	39.2	1.25	0.49
Select	7.2	6.3	0.63	0.29
No roll[2]	0.22	0.09	0.11	0.42
USDA yield grade, %				
YG 1	0.63	0.57	0.19	0.82
YG 2	19.6	15.5	0.94	0.002
YG 3	58.5	60.7	1.26	0.21
YG 4	20.4	19.8	1.02	0.69
YG 5	1.4	2.6	0.35	0.01
Natural Programs, %				
CAN[3]	48.3	47.1	1.28	0.52
SRN[4]	28.9	29.7	1.09	0.56

[1]*Megasphaera* = oral drench consisting of 100 ml of a solution containing 1.5×10^8 cfu/mL *M. elsdenii* strain NCIMB 41125; Control = no oral drench.
[2]No roll = not USDA graded.
[3]CAN = Certified Angus BeefNatural (Upper USDA Choice; naturally produced).
[4]SRN = Star Ranch® Natural (Lower USDA Choice, and USDA Select; naturally produced).

Table 4-6. Comparison of morbidity among feedlot cattle assigned to one of two treatments (Control or *Megasphaera*[1]) at initial processing.

	%, Incidence			
Type of Illness	Control	*Megasphaera*	SEM	*P*-value
BRD[2]	2.42	2.38	0.37	0.93
AIP[3]	0.11	0.11	0.09	0.99
Diphtheria	0.07	0.19	0.09	0.31

[1]*Megasphaera* = oral drench consisting of 100 ml of a solution containing 1.5×10^8 cfu/mL *M. elsdenii* strain NCIMB 41125; Control = no oral drench.
[2]Bovine respiratory disease
[3]Acute interstitial pneumonia

Table 4-7. Comparison of mortality among feedlot cattle assigned to one of two treatments (Control or *Megasphaera*[1]) at initial processing.

	Treatment			
Cause of Death	Control	*Megasphaera*	SEM	*P*-value
Heart failure	0.05	0	0.05	0.32
Bloat	0.19	0.19	0.11	0.99
Pneumonia	0.06	0	0.05	0.32
AIP[2]	0.09	0.28	0.12	0.25
Downer[3]	0.09	0.22	0.09	0.31
Undetermined[4]	0	0.12	0.06	0.16

[1]*Megasphaera* = oral drench consisting of 100 ml of a solution containing 1.5×10^8 cfu/mL *M. elsdenii* strain NCIMB 41125; Control = no oral drench.
[2] Acute interstitial pneumonia
[3]Cattle that had to be euthanized due to injury
[4]Cause of death could not be determined

Appendix A - Statistical Analyses

This section contains a set of statistical analyses that were performed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). These analyses were conducted at each time point in the experiment. Comparisons were made between the average of the steers receiving live doses of *M. elsdenii* strain NCIMB 41125 and the placebo. Tests for orthogonal contrasts also were made.

Table A-1. Comparison of ruminal pH of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[1]		
		Placebo	1.62×10^{9}	1.62×10^{9}	1.62×10^{9}	SEM	1	2	3
-24	1	6.9	6.9	6.7	7.0	0.19	0.98	0.44	0.46
0	2	7.4	7.4	7.3	7.4	0.09	0.93	0.63	0.59
2		7.0	7.0	6.9	7.0	0.07	0.83	0.75	0.49
4		6.6	6.6	6.5	6.8	0.13	0.32	0.49	0.19
6		5.8	5.9	5.5	6.0	0.36	0.85	0.52	0.46
8		5.4	5.5	5.0	5.6	0.27	0.91	0.32	0.38
24	3	5.2	5.9	5.6	6.0	0.34	0.21	0.66	0.32
26		5.2	5.9	5.8	6.0	0.29	0.10	0.45	0.29
28		5.2	5.6	5.9	6.0	0.29	0.09	0.60	0.27
30		5.2	5.6	5.9	5.9	0.28	0.09	0.47	0.34
32		5.3	5.7	6.1	6.0	0.28	0.08	0.39	0.42
48	4	5.6	5.6	6.0	5.8	0.22	0.29	0.72	0.68
50		5.6	5.9	5.9	5.8	0.18	0.56	0.38	0.96
52		5.6	5.7	5.9	5.7	0.17	0.53	0.43	0.89
54		5.6	5.6	5.7	5.5	0.15	0.93	0.39	0.46
56		5.8	5.8	5.9	5.7	0.16	0.70	0.41	0.39
72	5	6.0	5.9	5.5	5.7	0.16	0.09	0.46	0.55
74		5.7	5.8	5.4	5.9	0.12	0.90	0.14	0.13
76		5.6	5.8	5.6	5.8	0.11	0.34	0.65	0.15
78		5.7	5.9	5.5	5.8	0.13	0.64	0.81	0.79
80		5.7	6.0	5.7	5.8	0.12	0.82	0.24	0.63
120	7	5.8	6.2	6.3	5.9	0.16	0.39	0.03	0.46
122		5.7	5.9	6.0	5.7	0.19	0.71	0.11	0.41
124		5.6	5.7	5.9	5.6	0.17	0.91	0.25	0.39
126		5.6	5.6	5.8	5.6	0.19	0.91	0.75	0.77
128		5.4	5.6	5.7	5.5	0.14	0.54	0.28	0.84
240	12	5.7	6.0	5.6	5.9	0.17	0.78	0.75	0.34
242		5.6	5.5	5.4	5.6	0.17	0.77	0.35	0.61
244		5.5	5.3	5.5	5.5	0.09	0.67	0.37	0.59
246		5.3	5.4	5.4	5.4	0.10	0.37	0.87	0.41
248		5.2	5.3	5.2	5.4	0.11	0.16	0.85	0.13
360	17	7.4	7.2	7.1	7.0	0.10	0.005	0.83	0.02
362		6.6	6.6	6.4	6.6	0.10	0.48	0.25	0.61
364		6.0	5.8	5.8	6.2	0.20	0.57	0.08	0.13
366		5.6	5.2	5.3	5.6	0.18	0.72	0.10	0.25
368		5.2	5.1	5.0	5.4	0.15	0.29	0.17	0.08
388	18	5.2	5.1	4.9	5.3	0.13	0.74	0.12	0.35
532	24	5.4	5.2	5.1	5.1	0.16	0.20	0.39	0.53
700	31	5.5	5.3	5.1	5.5	0.15	0.66	0.08	0.34
964	42	5.2	5.3	5.4	5.3	0.10	0.63	0.70	0.56

[1]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. esldenii*).

Table A-2. Comparison of ruminal acetate[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	53.6	56.0	48.8	53.8	6.71	0.57	0.55	0.88
0	2	24.5	25.8	26.6	22.4	3.25	0.71	0.41	0.41
2		36.8	32.3	31.7	34.1	2.85	0.50	0.26	0.89
4		36.2	35.0	33.4	36.4	2.79	0.93	0.48	0.66
6		44.1	43.5	42.8	45.6	6.01	0.89	0.78	0.76
8		40.1	41.8	42.0	47.9	9.69	0.59	0.83	0.56
24	3	29.2	33.9	32.5	40.2	8.49	0.39	0.85	0.38
26		32.5	38.5	28.9	44.3	9.39	0.54	0.62	0.32
28		33.8	46.1	30.5	48.4	10.65	0.56	0.79	0.36
30		34.2	47.1	31.3	48.4	10.51	0.57	0.84	0.37
32		34.7	49.3	31.3	48.0	9.75	0.62	0.92	0.40
48	4	46.3	43.3	40.2	48.8	7.79	0.90	0.47	0.55
50		46.5	44.2	43.2	52.0	7.99	0.66	0.49	0.43
52		45.8	41.0	43.9	52.1	7.79	0.54	0.42	0.36
54		45.0	41.6	44.8	57.9	7.51	0.24	0.29	0.13
56		48.5	45.4	45.2	61.3	7.53	0.23	0.18	0.08
72	5	47.4	49.9	56.6	55.7	5.99	0.22	0.76	0.50
74		52.7	49.3	60.3	52.5	6.43	0.73	0.74	0.83
76		52.3	49.0	52.2	52.7	5.21	0.85	0.70	0.79
78		51.4	47.1	52.2	52.7	4.55	0.66	0.60	0.64
80		50.6	44.9	47.4	48.1	4.07	0.79	0.45	0.93
120	7	44.5	42.6	35.9	48.7	2.89	0.64	0.02	0.03
122		48.2	44.9	38.2	48.9	2.99	0.74	0.04	0.16
124		54.1	52.4	43.8	54.0	4.93	0.69	0.25	0.51
126		47.9	50.8	44.8	48.5	3.73	0.79	0.92	0.89
128		44.2	47.1	41.1	45.6	3.84	0.92	0.84	0.75
240	12	43.2	37.5	40.8	41.8	3.25	0.93	0.26	0.71
242		49.5	48.8	44.3	54.0	4.34	0.62	0.20	0.17
244		54.5	54.8	43.0	54.7	4.57	0.49	0.13	0.35
246		57.8	49.7	45.0	61.5	6.79	0.81	0.05	0.13
248		53.3	48.0	44.8	49.7	4.37	0.35	0.13	0.78
360	17	15.2	16.8	19.5	27.4	2.75	0.007	0.28	0.007
362		26.7	26.1	32.0	37.5	3.32	0.01	0.32	0.02
364		35.7	35.9	38.0	38.3	4.09	0.58	0.99	0.70
366		38.1	35.3	39.1	41.1	3.61	0.44	0.53	0.41
368		42.6	41.3	47.6	53.1	4.10	0.05	0.39	0.06
388	18	44.4	35.8	39.8	33.7	3.64	0.09	0.91	0.13
532	24	37.6	31.4	37.6	38.9	3.05	0.46	0.34	0.39
700	31	45.9	37.4	39.0	39.0	3.68	0.21	0.51	0.32

[1]Concentrations are expressed in *mM*.
[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. elsdenii*).

Table A-3. Comparison of ruminal propionate[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	13.0	12.9	10.6	12.0	1.75	0.51	0.66	0.94
0	2	4.6	4.9	5.5	3.9	0.87	0.72	0.29	0.29
2		9.4	7.8	8.0	8.6	0.92	0.61	0.27	0.87
4		10.7	10.0	10.1	10.8	0.99	0.93	0.50	0.66
6		16.0	14.6	15.4	16.7	2.64	0.79	0.61	0.64
8		16.2	15.1	17.3	20.3	4.53	0.48	0.66	0.45
24	3	17.4	17.1	28.0	19.6	4.55	0.39	0.38	0.82
26		18.0	19.2	21.7	20.2	4.66	0.66	0.77	0.91
28		17.3	23.4	20.9	21.4	5.00	0.67	0.58	0.88
30		16.2	23.6	21.2	21.0	4.80	0.58	0.44	0.91
32		15.9	27.6	21.1	21.2	4.21	0.63	0.20	0.95
48	4	23.7	21.9	21.7	20.4	3.27	0.50	0.95	0.59
50		22.1	22.1	21.9	20.7	3.58	0.79	0.87	0.75
52		20.9	20.2	20.6	21.0	3.57	0.97	0.87	0.92
54		20.1	20.5	19.6	24.7	3.78	0.45	0.55	0.31
56		20.6	22.0	20.6	27.2	4.01	0.32	0.52	0.21
72	5	19.8	22.4	21.0	27.1	2.95	0.15	0.56	0.10
74		22.2	22.4	22.2	26.0	3.45	0.48	0.61	0.36
76		22.2	22.5	19.1	26.3	2.93	0.51	0.26	0.16
78		21.2	21.7	19.2	27.3	3.03	0.27	0.23	0.08
80		19.9	20.3	17.2	25.1	3.24	0.41	0.27	0.14
120	7	25.5	21.3	18.9	27.9	3.85	0.77	0.11	0.19
122		27.3	25.0	20.8	30.8	3.77	0.71	0.12	0.16
124		34.6	30.6	25.1	35.3	4.69	0.87	0.16	0.36
126		31.5	31.2	26.5	34.0	4.46	0.88	0.40	0.42
128		30.5	29.7	24.9	34.2	4.54	0.76	0.29	0.29
240	12	33.6	29.5	37.2	37.0	4.10	0.34	0.63	0.46
242		37.8	37.4	40.9	48.4	5.22	0.15	0.46	0.13
244		42.7	43.5	39.4	49.0	4.70	0.49	0.36	0.21
246		47.6	40.7	42.0	59.4	6.86	0.25	0.10	0.07
248		46.4	40.5	43.3	49.6	4.46	0.54	0.19	0.25
360	17	5.9	7.0	9.5	10.2	1.68	0.06	0.90	0.18
362		14.5	11.6	17.5	15.1	2.25	0.38	0.91	0.81
364		23.3	23.8	25.2	16.5	4.09	0.31	0.27	0.12
366		26.7	26.8	27.7	20.2	3.79	0.29	0.33	0.14
368		30.5	34.5	35.0	33.2	4.79	0.69	0.56	0.98
532	24	47.1	44.9	38.6	43.2	4.54	0.39	0.48	0.95
700	31	33.7	38.4	35.5	31.6	4.78	0.67	0.42	0.47
964	42	43.7	28.6	27.9	36.4	3.99	0.25	0.03	0.55

[1]Concentrations are expressed in *mM*.
[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. elsdenii*).

Table A-4. Comparison of ruminal A:P of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[1]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	4.3	4.4	4.3	4.5	0.13	0.48	0.48	0.25
0	2	5.5	5.7	4.9	5.8	0.42	0.90	0.46	0.38
2		3.9	4.1	3.9	4.0	0.16	0.92	0.53	0.85
4		3.3	3.5	3.3	3.3	0.13	0.69	0.59	0.74
6		2.8	3.1	2.8	2.7	0.15	0.30	0.28	0.24
8		3.2	3.0	2.7	2.3	0.39	0.13	0.80	0.19
24	3	9.6	1.9	1.1	2.0	3.57	0.16	0.25	0.60
26		17.2	1.9	1.3	2.1	6.93	0.16	0.26	0.57
28		35.8	1.8	1.4	2.2	15.7	0.17	0.29	0.56
30		26.8	1.8	1.4	2.2	10.48	0.14	0.24	0.53
32		31.5	1.7	1.4	2.2	13.07	0.16	0.26	0.55
48	4	1.9	2.1	1.7	2.4	0.26	0.33	0.33	0.10
50		2.1	2.3	1.8	2.5	0.29	0.56	0.55	0.26
52		2.2	2.4	2.0	2.4	0.27	0.78	0.73	0.49
54		2.3	2.3	2.2	2.3	0.25	0.88	0.78	0.91
56		2.5	2.3	2.1	2.2	0.27	0.49	0.63	0.84
72	5	2.3	2.2	2.7	2.1	0.25	0.96	0.34	0.35
74		2.3	2.2	2.8	2.1	0.27	0.94	0.31	0.31
76		2.3	2.2	2.8	2.1	0.29	0.95	0.33	0.32
78		2.4	2.2	2.8	2.1	0.31	0.84	0.41	0.32
80		2.6	2.3	2.9	2.2	0.35	0.73	0.58	0.36
120	7	1.7	2.1	1.9	2.0	0.25	0.56	0.57	0.74
122		1.8	1.8	1.8	1.8	0.23	0.85	0.82	0.98
124		1.5	1.7	1.8	1.7	0.21	0.47	0.58	0.78
126		1.5	1.6	1.7	1.6	0.18	0.56	0.61	0.86
128		1.4	1.6	1.6	1.5	0.16	0.64	0.47	0.94
240	12	1.3	1.3	1.1	1.2	0.14	0.39	0.77	0.77
242		1.3	1.3	1.1	1.2	0.17	0.40	0.57	0.92
244		1.3	1.2	1.1	1.1	0.16	0.37	0.92	0.59
246		1.2	1.2	1.1	1.0	0.14	0.26	0.72	0.30
248		1.1	1.2	1.0	1.0	0.12	0.26	0.59	0.27
360	17	2.9	2.6	2.1	3.0	0.44	0.87	0.16	0.36
362		1.9	2.3	1.8	2.7	0.29	0.15	0.27	0.04
364		1.7	1.6	1.5	2.6	0.31	0.08	0.10	0.02
366		1.5	1.4	1.4	2.2	0.26	0.09	0.12	0.02
368		1.4	1.3	1.3	1.8	0.23	0.30	0.24	0.12
532	24	0.9	0.7	1.0	0.7	0.07	0.30	0.43	0.07
700	31	1.2	0.8	1.0	1.4	0.23	0.42	0.15	0.18
964	42	1.1	1.3	1.4	1.1	0.18	0.94	0.15	0.33

[1]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. elsdenii*).

Table A-5. Comparison of ruminal butyrate[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	6.5	6.2	5.6	5.8	0.94	0.53	0.76	0.79
0	2	2.5	2.3	3.1	2.0	0.44	0.78	0.29	0.24
2		5.7	4.6	5.1	5.3	0.67	0.76	0.34	0.87
4		6.6	5.9	6.2	6.1	0.83	0.78	0.70	0.94
6		9.4	8.0	9.1	9.3	1.82	0.89	0.66	0.80
8		9.3	7.9	9.7	12.0	3.02	0.48	0.55	0.40
24	3	13.3	9.5	15.9	19.3	4.45	0.24	0.44	0.24
26		13.7	11.6	15.6	18.9	3.94	0.28	0.50	0.26
28		13.4	13.3	17.1	20.3	3.89	0.18	0.67	0.22
30		12.0	13.4	17.2	21.0	3.49	0.06	0.72	0.10
32		11.7	14.4	16.7	22.5	3.51	0.05	0.67	0.07
48	4	19.6	22.6	15.3	22.1	3.93	0.99	0.63	0.53
50		20.4	20.9	15.4	21.8	3.47	0.94	0.41	0.48
52		21.9	19.4	15.6	20.8	3.46	0.66	0.29	0.66
54		22.6	19.7	17.2	23.6	3.24	0.97	0.18	0.33
56		23.0	19.9	16.1	24.4	2.92	0.98	0.08	0.19
72	5	19.8	19.8	22.3	23.3	3.04	0.35	0..86	0.45
74		22.3	20.6	23.7	21.4	3.10	0.99	0.93	0.82
76		22.5	21.4	21.2	22.3	2.39	0.95	0.66	0.83
78		22.1	20.7	22.5	24.4	2.71	0.49	0.56	0.42
80		20.5	19.5	20.8	22.2	2.57	0.59	0.64	0.53
120	7	16.5	12.3	14.3	20.8	2.90	0.27	0.09	0.08
122		19.2	14.3	15.0	22.8	3.16	0.43	0.07	0.09
124		19.6	16.7	16.6	26.1	4.12	0.32	0.16	0.10
126		17.4	17.3	17.7	25.2	4.03	0.21	0.36	0.12
128		16.6	16.5	16.1	23.9	3.43	0.19	0.28	0.08
240	12	8.8	6.56	8.0	7.3	1.81	0.71	0.68	0.81
242		10.6	9.0	8.3	10.2	2.29	0.85	0.47	0.74
244		11.5	10.1	7.4	11.2	2.42	0.74	0.31	0.59
246		13.3	9.8	7.9	13.4	2.58	0.87	0.09	0.32
248		14.0	9.3	8.2	11.5	2.93	0.52	0.19	0.77
360	17	1.7	2.1	2.5	3.2	0.44	0.02	0.70	0.04
362		4.2	3.6	4.3	6.3	0.67	0.03	0.05	0.007
364		6.6	6.0	5.2	7.5	1.04	0.67	0.17	0.19
366		8.8	7.6	5.6	9.7	1.35	0.91	0.07	0.14
368		11.5	12.1	9.0	14.6	2.06	0.52	0.24	0.14
532	24	11.3	15.7	15.2	17.5	1.98	0.07	0.59	0.17
700	31	11.9	8.1	9.5	10.4	1.95	0.76	0.37	0.89
964	42	8.3	8.9	8.9	16.6	1.80	0.01	0.22	0.008

[1]Concentrations are expressed in *mM*.
[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. elsdenii*).

Table A-6. Comparison of ruminal isobutyrate[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	1.50	1.45	1.19	1.49	0.12	0.62	0.16	0.42
0	2	1.03	1.04	1.03	1.01	0.11	0.88	0.95	0.87
2		1.09	1.02	1.01	1.07	0.10	0.84	0.50	0.80
4		0.96	1.05	0.91	1.09	0.10	0.59	0.65	0.33
6		0.91	0.93	0.93	0.99	0.13	0.70	0.84	0.65
8		0.66	0.78	0.76	0.89	0.19	0.43	0.95	0.46
24	3	0.41	0.47	0.37	0.81	0.19	0.21	0.33	0.09
26		0.50	0.50	0.52	0.81	0.17	0.20	0.38	0.12
28		0.41	0.52	0.38	0.79	0.17	0.23	0.40	0.10
30		0.37	0.51	0.43	0.75	0.16	0.18	0.56	0.12
32		0.38	0.54	0.42	0.75	0.16	0.18	0.58	0.12
48	4	0.54	0.61	0.52	0.97	0.15	0.08	0.19	0.03
50		0.51	0.64	0.48	0.88	0.14	0.14	0.36	0.05
52		0.49	0.53	0.45	0.73	0.12	0.26	0.34	0.11
54		0.54	0.49	0.45	0.78	0.11	0.19	0.10	0.04
56		0.56	0.51	0.36	0.74	0.09	0.35	0.02	0.02
72	5	0.61	0.73	0.53	0.77	0.10	0.56	0.56	0.24
74		0.64	0.67	0.52	0.69	0.13	0.99	0.57	0.58
76		0.60	0.65	0.44	0.63	0.10	0.80	0.48	0.55
78		0.56	0.59	0.44	0.62	0.08	0.98	0.39	0.39
80		0.56	0.62	0.52	0.56	0.08	0.74	0.93	0.93
120	7	0.63	0.69	0.69	0.66	0.11	0.88	0.67	0.91
122		0.74	0.78	0.75	0.72	0.10	0.84	0.73	0.76
124		0.75	0.91	0.80	0.73	0.11	0.72	0.26	0.45
126		0.61	0.63	0.68	0.65	0.09	0.64	0.74	0.94
128		0.57	0.53	0.57	0.59	0.09	0.75	0.60	0.63
240	12	0.54	0.52	0.41	0.59	0.12	0.96	0.42	0.49
242		0.54	0.49	0.38	0.62	0.11	0.81	0.24	0.29
244		0.53	0.43	0.33	0.58	0.10	0.88	0.11	0.22
246		0.55	0.38	0.31	0.66	0.11	0.56	0.03	0.06
248		0.48	0.33	0.31	0.54	0.10	0.70	0.05	0.13
360	17	0.74	0.71	0.93	1.37	0.15	0.01	0.15	0.01
362		0.61	0.57	0.83	1.17	0.17	0.02	0.28	0.02
364		0.44	0.37	0.49	0.85	0.13	0.04	0.13	0.02
366		0.28	0.23	0.31	0.70	0.13	0.04	0.12	0.01
368		0.27	0.25	0.28	0.71	0.09	0.01	0.03	0.002
532	24	0.61	0.43	0.49	0.47	0.08	0.29	0.43	0.58
700	31	0.53	0.33	0.39	0.55	0.10	0.81	0.08	0.22
964	42	0.36	0.32	0.37	0.62	0.07	0.02	0.09	0.01

[1]Concentrations are expressed in m*M*.
[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. elsdenii*).

Table A-7. Comparison of ruminal isovalerate[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	1.95	1.98	1.63	0.93	0.21	0.67	0.53	0.75
0	2	1.29	1.29	1.29	1.26	0.15	0.86	0.93	0.84
2		1.12	1.05	1.01	1.15	0.11	0.93	0.37	0.50
4		0.70	0.75	0.68	0.84	0.10	0.44	0.57	0.27
6		0.43	0.49	0.51	0.48	0.10	0.72	0.65	1.0
8		0.22	0.35	0.37	0.31	0.11	0.58	0.41	0.97
24	3	0.68	0.56	0.43	0.88	0.24	0.23	0.59	0.16
26		0.39	0.54	0.37	0.81	0.23	0.33	0.54	0.19
28		0.40	0.56	0.54	0.83	0.23	0.23	0.77	0.22
30		0.39	0.56	0.46	0.79	0.25	0.33	0.72	0.27
32		0.42	0.56	0.39	0.70	0.25	0.52	0.73	0.38
48	4	0.67	0.72	0.48	1.00	0.27	0.53	0.39	0.24
50		0.55	0.59	0.42	0.83	0.22	0.52	0.43	0.26
52		0.55	0.50	0.43	0.64	0.19	0.82	0.51	0.52
54		0.61	0.48	0.43	0.69	0.19	0.85	0.33	0.44
56		0.72	0.56	0.17	0.64	0.16	0.31	0.03	0.32
72	5	0.82	0.82	0.49	0.93	0.28	0.99	0.45	0.50
74		0.90	0.64	0.49	0.71	0.32	0.62	0.47	0.94
76		0.73	0.61	0.35	0.64	0.22	0.60	0.37	0.76
78		0.69	0.57	0.40	0.62	0.22	0.69	0.46	0.81
80		0.67	0.63	0.41	0.63	0.21	0.73	0.55	0.79
120	7	0.87	0.85	0.95	0.89	0.33	0.91	0.95	0.99
122		0.80	0.69	0.89	0.84	0.29	0.81	0.91	0.88
124		0.81	0.63	0.82	0.81	0.29	0.89	0.79	0.88
126		0.75	0.54	0.77	0.79	0.30	0.78	0.69	0.75
128		0.69	0.48	0.63	0.74	0.25	0.81	0.53	0.65
240	12	0.69	0.96	0.53	1.06	0.25	0.56	0.62	0.28
242		0.62	0.75	0.42	1.13	0.23	0.25	0.21	0.06
244		0.58	0.62	0.38	1.14	0.24	0.18	0.14	0.14
246		0.61	0.56	0.33	1.25	0.23	0.12	0.05	0.01
248		0.51	0.51	0.32	1.02	0.20	0.12	0.08	0.02
360	17	0.95	1.04	1.52	2.92	0.33	0.001	0.07	0.001
362		0.65	0.78	1.39	2.28	0.45	0.01	0.38	0.02
364		0.37	0.52	0.79	1.79	0.32	0.007	0.18	0.004
366		0.31	0.36	0.73	1.45	0.33	0.02	0.29	0.02
368		0.46	0.31	0.84	1.33	0.30	0.04	0.31	0.04
532	24	0.87	0.63	0.74	0.64	0.20	0.48	0.85	0.59
700	31	1.74	0.40	0.36	1.35	0.73	0.68	0.11	0.50
964	42	0.96	0.51	0.45	0.75	0.35	0.68	0.36	0.79

[1]Concentrations are expressed in m*M*.

[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. elsdenii*).

Table A-8. Comparison of ruminal valerate[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	0.93	0.92	0.71	0.85	0.16	0.52	0.64	0.97
0	2	0.39	0.35	0.49	0.32	0.07	0.79	0.39	0.29
2		1.05	0.79	0.84	1.17	0.14	0.52	0.05	0.11
4		1.13	1.00	0.91	1.25	0.19	0.76	0.24	0.30
6		1.13	1.05	1.09	1.29	0.25	0.66	0.59	0.52
8		0.85	0.85	0.93	1.30	0.32	0.33	0.56	0.27
24	3	0.78	2.76	10.04	5.53	1.67	0.01	0.08	0.61
26		0.93	3.31	11.13	5.37	1.90	0.03	0.05	0.91
28		0.91	3.47	12.30	5.09	1.96	0.03	0.03	0.84
30		0.87	3.31	11.92	4.54	1.82	0.03	0.02	0.70
32		0.83	3.21	11.59	4.55	1.95	0.05	0.03	0.77
48	4	4.70	6.79	6.49	3.36	2.48	0.70	0.31	0.38
50		5.15	5.75	5.54	3.09	1.94	0.48	0.45	0.31
52		5.19	4.90	4.89	2.74	1.77	0.37	0.61	0.29
54		5.11	4.57	4.75	3.16	1.69	0.47	0.76	0.41
56		4.55	3.99	5.43	3.09	1.43	0.64	0.53	0.35
72	5	2.40	2.83	2.84	2.78	0.71	0.72	0.73	0.92
74		2.67	2.97	2.83	2.42	0.58	0.74	0.55	0.56
76		2.46	2.88	2.59	2.39	0.57	0.85	0.59	0.71
78		2.18	2.63	2.62	2.45	0.56	0.75	0.59	0.97
80		1.98	2.34	2.26	2.16	0.49	0.84	0.65	0.96
120	7	2.91	2.12	2.44	2.12	0.61	0.46	0.71	0.61
122		3.27	2.86	2.99	2.36	0.75	0.45	0.88	0.45
124		3.25	3.09	3.10	2.58	0.87	0.61	0.84	0.58
126		3.00	3.16	3.05	2.32	0.86	0.58	0.62	0.46
128		3.00	2.78	2.81	2.22	0.73	0.49	0.81	0.46
240	12	3.85	2.36	3.81	2.24	1.08	0.50	0.97	0.39
242		4.42	3.81	4.33	3.03	1.49	0.59	0.82	0.51
244		4.78	4.07	3.49	2.87	1.30	0.30	0.98	0.42
246		5.26	3.74	3.61	3.31	1.19	0.29	0.62	0.53
248		4.67	3.41	3.74	2.75	1.24	0.35	0.91	0.42
360	17	0.48	0.49	0.99	1.25	0.19	0.01	0.54	0.02
362		1.54	0.95	1.81	2.32	0.42	0.11	0.21	0.09
364		2.49	1.50	2.17	1.99	0.54	0.74	0.46	0.93
366		2.92	2.10	2.57	2.31	0.72	0.68	0.71	0.79
368		3.02	3.94	3.25	3.87	0.97	0.67	0.88	0.68
532	24	3.58	5.29	9.64	6.21	1.70	0.14	0.17	0.97
700	31	2.47	34.22	5.26	3.26	1.10	0.47	0.11	0.55
964	42	2.63	4.10	4.81	4.58	1.50	0.36	0.62	0.69

[1]Concentrations are expressed in m*M*.
[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. elsdenii*).

Table A-9. Comparison of ruminal total VFA of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[1]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	80.4	79.5	65.7	76.1	9.78	0.55	0.58	0.94
0	2	34.4	35.8	38.2	31.1	4.81	0.73	0.39	0.38
2		55.3	47.8	47.8	51.5	5.69	0.59	0.25	0.83
4		56.4	53.9	52.4	56.6	4.85	0.96	0.49	0.68
6		72.0	68.6	69.9	74.6	10.70	0.85	0.71	0.73
8		67.5	66.9	71.2	82.9	17.51	0.53	0.73	0.49
24	3	61.6	64.5	87.5	86.5	16.62	0.21	0.91	0.44
26		66.1	73.9	78.4	90.6	17.38	0.33	0.89	0.39
28		66.4	87.5	81.8	96.9	19.13	0.33	0.88	0.41
30		64.2	88.6	82.6	96.6	18.51	0.27	0.77	0.39
32		64.1	95.7	81.7	97.9	16.71	0.26	0.65	0.38
48	4	95.6	96.0	84.8	96.8	10.70	0.87	0.60	0.72
50		95.3	94.3	87.1	99.5	10.73	0.91	0.54	0.57
52		94.9	86.7	86.0	98.2	10.86	0.86	0.36	0.49
54		94.1	87.4	87.4	110.9	10.39	0.29	0.17	0.10
56		98.1	92.5	88.0	117.6	10.28	0.27	0.11	0.06
72	5	91.0	96.6	103.9	110.7	9.81	0.14	0.95	0.24
74		101.6	96.7	110.1	103.8	11.26	0.70	0.95	0.94
76		100.9	97.1	96.0	105.1	8.12	0.76	0.44	0.47
78		98.3	93.4	97.5	108.2	7.68	0.34	0.33	0.21
80		94.4	88.4	88.8	98.9	7.23	0.68	0.29	0.34
120	7	91.0	80.1	73.4	101.2	5.11	0.21	0.0004	0.01
122		99.6	88.7	78.7	106.5	4.90	0.61	0.001	0.01
124		113.3	104.4	90.4	119.6	9.62	0.91	0.07	0.15
126		101.3	103.8	93.7	111.6	7.61	0.55	0.33	0.19
128		95.7	97.3	86.4	107.4	6.98	0.45	0.19	0.10
240	12	90.8	77.5	90.8	90.1	5.99	0.69	0.31	0.60
242		103.6	100.4	98.8	117.5	8.62	0.32	0.23	0.12
244		114.8	113.7	94.1	119.7	7.86	0.89	0.11	0.19
246		125.3	105.0	99.3	139.6	13.14	0.52	0.03	0.06
248		119.5	102.2	100.7	115.3	8.86	0.67	0.05	0.36
360	17	25.1	28.3	35.2	46.5	5.10	0.01	0.44	0.01
362		48.4	43.7	58.0	64.9	6.60	0.03	0.34	0.05
364		68.9	68.3	72.1	67.1	8.72	0.96	0.80	0.79
366		77.2	72.6	76.2	75.6	7.68	0.97	0.80	0.98
368		88.5	92.5	96.1	106.9	8.40	0.13	0.68	0.14
532	24	108.1	102.9	104.5	101.9	8.36	0.63	0.97	0.70
700	31	88.0	82.8	88.8	86.2	7.31	0.98	0.98	0.90
964	42	102.1	80.0	81.6	98.2	7.11	0.82	0.06	0.35

[1]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. elsdenii*).

Table A-10. Comparison of ruminal lactate[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	-	-	-	-	-	-	-	-
0	2	-	-	-	-	-	-	-	-
2		5.067	3.65	4.71	3.42	1.43	0.55	0.97	0.53
4		15.81	18.90	20.03	7.25	9.49	0.57	0.42	0.34
6		29.26	29.92	51.33	20.26	16.56	0.94	0.36	0.40
8		34.42	46.84	64.63	33.86	19.68	0.86	0.29	0.53
24	3	49.77	24.56	3.53	3.03	19.17	0.08	0.53	0.32
26		15.50	15.08	5.57	4.53	8.23	0.27	0.97	0.44
28		43.24	27.29	2.10	1.72	18.71	0.10	0.68	0.31
30		51.73	25.17	2.50	2.18	19.99	0.08	0.52	0.31
32		34.70	18.98	3.18	2.60	13.85	0.10	0.59	0.33
48	4	0.41	0.78	0.55	0.43	0.29	0.88	0.41	0.65
50		0.10	0.16	0.40	0.40	0.14	0.10	0.83	0.29
52		0.11	0.08	0.41	0.23	0.14	0.30	0.64	0.84
54		0.06	0.17	0.23	0.18	0.10	0.39	0.44	0.84
56		0.04	0.16	0.46	0.32	0.10	0.01	0.16	0.35
72	5	0.13	0.04	0.37	0.05	0.10	0.85	0.23	0.24
74		0.19	0.19	0.15	0.03	0.06	0.08	0.36	0.06
76		0.24	0.20	0.22	0.07	0.06	0.12	0.41	0.07
78		0.21	0.13	0.19	0.11	0.06	0.44	0.92	0.45
80		0.22	0.12	0.16	0.10	0.08	0.39	0.76	0.51
120	7	0.28	0.11	0.10	0.29	0.09	0.99	0.07	0.25
122		0.55	0.40	0.09	0.16	0.23	0.17	0.63	0.49
124		1.19	0.77	0.18	0.27	0.56	0.20	0.66	0.20
126		0.44	0.58	0.20	0.34	0.24	0.53	0.99	0.80
128		1.02	0.50	0.39	0.29	0.28	0.09	0.48	0.30
240	12	0.10	0.24	0.11	0.03	0.10	0.46	0.30	0.32
242		0.14	0.30	0.22	0.22	0.10	0.70	0.44	0.98
244		0.23	0.44	0.15	0.37	0.20	0.89	0.96	0.68
246		0.26	0.39	0.16	0.22	0.11	0.47	0.76	0.68
248		0.26	0.48	0.43	0.98	0.41	0.27	0.69	0.24
360	17	0.42	0.26	0.33	0.03	0.12	0.06	0.59	0.04
362		12.66	11.29	13.05	1.35	3.45	0.06	0.16	0.02
364		10.36	17.87	16.37	2.23	7.88	0.48	0.19	0.19
366		3.36	8.61	8.77	1.56	4.32	0.79	0.17	0.30
368		4.29	0.79	4.00	1.43	2.59	0.65	0.86	0.60
532	24	0.53	0.60	0.54	0.59	0.19	0.88	0.97	0.87
700	31	0.04	0.11	0.18	0.19	0.07	0.11	0.67	0.32
964	42	0.16	0.22	0.30	0.03	0.07	0.32	0.05	0.04

[1]Concentrations are expressed in m*M*.
[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. elsdenii*).

Table A-11. Comparison of total ruminal bacterial genomes[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	10.09	9.84	9.70	9.79	0.19	0.25	0.39	0.69
0	2	10.08	9.77	9.76	10.19	0.16	0.67	0.04	0.11
2		9.60	9.89	9.59	10.01	0.13	0.12	0.61	0.05
4		9.48	9.14	9.68	10.05	0.31	0.04	0.14	0.03
6		9.90	9.86	9.90	9.82	0.21	0.79	0.92	0.75
8		9.80	10.16	9.65	10.03	0.15	0.77	0.97	0.35
24	3	10.18	9.91	9.57	10.06	0.27	0.57	0.18	0.59
28		9.97	10.12	9.57	10.51	0.17	0.18	0.04	0.01
32		9.92	9.99	9.57	10.1	0.16	0.88	0.19	0.18
72	5	9.73	9.70	9.84	10.18	0.29	0.27	0.53	0.23
76		9.85	9.96	9.52	10.18	0.19	0.53	0.18	0.09
80		9.60	9.65	9.60	9.83	0.19	0.48	0.63	0.35
122	7	9.98	10.11	9.82	10.14	0.25	0.83	0.75	0.55
126		9.92	10.08	9.88	10.02	0.23	0.92	0.94	0.83
240	12	10.03	9.95	10.12	10.32	0.12	0.11	0.23	0.07
244		9.72	9.75	9.64	9.73	0.17	0.92	0.86	0.89
360	17	10.02	10.66	9.98	9.88	0.21	0.27	0.10	0.19
364		10.49	9.92	10.22	10.33	0.18	0.77	0.08	0.59
368		10.21	9.98	10.24	10.39	0.19	0.33	0.32	0.26
532	24	10.10	9.22	9.38	9.25	0.21	0.03	0.13	0.24
700	31	9.97	9.41	9.70	9.31	0.28	0.22	0.77	0.28
964	42	9.42	9.58	9.28	9.46	0.12	0.83	0.91	0.92

[1]Populations are expressed in genomes/mL $\log_{10}$.
[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. esldenii*).

Table A-12. Comparison of undifferentiated ruminal *Megasphaera elsdenii* populations[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	4.73	4.51	4.53	4.99	0.20	0.38	0.12	0.11
0	2	4.65	4.42	4.91	4.74	0.24	0.47	0.88	0.75
2		4.54	5.69	6.38	7.58	0.19	< 0.0001	0.91	< 0.0001
4		4.47	5.60	6.58	7.91	0.20	< 0.0001	0.62	< 0.0001
6		4.74	5.73	7.12	7.84	0.28	< 0.0001	0.64	< 0.0001
8		4.79	5.86	6.9	7.94	0.16	< 0.0001	0.93	< 0.0001
24	3	4.97	7.21	8.30	7.98	0.45	0.0003	0.015	0.04
28		5.10	7.50	8.40	8.33	0.49	0.0004	0.03	0.04
32		5.35	7.42	8.33	7.96	0.47	0.0013	0.02	0.11
72	5	6.36	7.27	7.64	7.67	0.62	0.15	0.49	0.44
76		6.65	7.56	7.26	7.61	0.61	0.36	0.66	0.53
80		6.59	7.40	7.24	7.11	0.65	0.62	0.46	0.96
122	7	6.11	7.52	6.96	6.33	0.57	0.88	0.14	0.39
126		5.82	7.09	6.90	5.99	0.56	0.88	0.04	0.28
240	12	7.46	7.14	7.46	6.75	0.39	0.33	0.63	0.22
244		7.11	7.40	7.07	6.62	0.52	0.54	0.72	0.46
360	17	6.90	7.28	6.90	6.80	0.36	0.68	0.52	0.59
364		7.19	6.77	7.28	6.78	0.52	0.77	0.94	0.63
368		7.69	7.34	7.96	7.18	0.46	0.67	0.65	0.38
532	24	7.97	7.02	7.52	7.23	0.27	0.17	0.14	0.43
700	31	6.70	6.26	6.91	6.10	0.47	0.59	0.87	0.40
964	42	5.42	6.16	6.20	5.39	0.46	0.99	0.19	0.38

[1]Populations are expressed in genomes/mL $\log_{10}$.
[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. esldenii*).

Table A-13. Comparison of total ruminal *Megasphaera elsdenii* strain NCIMB 41125 populations[1] of cattle intraruminally dosed with a placebo or 1.62×10^{9}, 1.62×10^{10}, or 1.62×10^{11} CFU of *M. elsdenii* strain NCIMB 41125.

post-inoculation (h, d)		CFU of *M. elsdenii* NCIMB 41125					*P*-value[2]		
		Placebo	1.62×10^{9}	1.62×10^{10}	1.62×10^{11}	SEM	1	2	3
-24	1	4.58	4.44	4.50	4.9	0.18	0.23	0.15	0.08
0	2	4.51	4.42	4.76	4.44	0.17	0.85	0.49	0.52
2		4.42	4.98	6.10	6.91	0.21	< 0.0001	0.55	< 0.0001
4		4.42	5.11	6.22	7.23	0.20	< 0.0001	0.43	< 0.0001
6		4.43	5.55	6.65	6.81	0.26	< 0.0001	0.09	0.0013
8		4.62	5.76	6.86	7.67	0.19	< 0.0001	0.36	< 0.0001
24	3	4.78	7.11	8.16	7.69	0.44	0.0004	0.008	0.07
28		4.97	7.38	8.34	8.09	0.49	0.0005	0.02	0.06
32		5.27	7.36	8.14	7.54	0.55	0.009	0.03	0.35
72	5	6.00	6.74	7.21	7.28	0.60	0.13	0.59	0.38
76		6.21	7.12	6.65	7.08	0.61	0.44	0.70	0.56
80		5.92	7.03	6.61	6.78	0.61	0.44	0.45	0.72
122	7	5.76	7.28	6.63	6.29	0.53	0.68	0.09	0.66
126		5.59	6.87	6.79	5.78	0.52	0.81	0.03	0.24
240	12	6.02	6.45	6.94	6.01	0.39	0.80	0.10	0.32
244		6.01	6.37	6.33	5.75	0.46	0.69	0.32	0.37
360	17	5.93	6.53	6.8	6.42	0.29	0.16	0.08	0.99
364		6.21	6.06	6.69	6.21	0.46	0.77	0.73	0.84
368		6.75	6.53	7.35	6.49	0.42	0.99	0.46	0.43
532	24	6.74	5.93	5.86	5.68	0.26	0.02	0.19	0.16
700	31	5.33	5.11	5.59	5.01	0.35	0.79	0.81	0.53
964	42	4.65	4.84	4.62	4.52	0.17	0.35	0.79	0.49

[1]Populations are expressed in genomes/mL $\log_{10}$.
[2]Contrasts (1: linear, 2: quadratic, 3: Placebo vs. *M. esldenii*).