Ruminal characteristics and feedlot performance of steers during accelerated step-up to high-concentrate diets using *Megasphaera elsdenii* (Lactipro *advance*).

by

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Abstract

Ruminal characteristics and feedlot performance were measured for steers adapted to a high-grain diet using a traditional 22-d step-up program (Control) and for steers adapted over 10 d with the aid of *M. elsdenii* NCIMB 41125 fresh culture administered as an oral gavage (Fresh), an oral gavage of rehydrated lyophilized culture (Rehyd), or Rehyd combined with lyophilized culture powder administered daily as a ration top-dress (Rehyd+Daily). Yearling steers (n = 435; initial BW = 408 ± 5 kg) were blocked by weight and randomly allocated to 64 concrete surfaced pens with 7 steers/pen and 16 pens/treatment. Step-up diets contained 40, 30, and 20% corn silage, and were followed by a finishing diet containing 10% corn silage and 90% concentrate. Ruminal fluid was obtained from a subset of steers by rumenocentesis 26 h after their first feeding, and inoculated into culture tubes containing lactate medium to determine differences in capacity for lactate metabolism over 24 h. A radiofrequency pH bolus was placed in the reticulorumen of 32 steers, that measured ruminal pH every 10 min for 124 d. Steers were fed once daily *ad libitum* for 156 d, then weighed, and transported 450 km to a commercial abattoir for harvest. HCW and incidence of liver abscesses were determined at harvest and carcass traits were evaluated after 36 h of refrigeration. No differences were detected for feedlot performance ($P > 0.20$), liver abscesses ($P = 0.45$), or carcass traits ($P > 0.20$). Capacity for lactate utilization was increased with all forms of *M. elsdenii*, as evidenced by increases in optical density (absorbance) of *M. elsdenii* cultures, disappearance of lactate, and increase in butyrate production ($P < 0.01$). Steers on Rehyd and Rehyd+Daily treatments spent less time between pH thresholds of $5.6 > pH > 5.2$ ($P < 0.01$); $5.2 > pH > 5.0$ ($P < 0.01$); and $pH < 5.0$ ($P < 0.01$) than Controls throughout the finishing period. In conclusion, steers dosed with *M. elsdenii* can be stepped up to finishing diets in 10 d with no adverse effects on performance. Ruminal fluid containing fresh cultures or freeze-dried and rehydrated cultures of *M. elsdenii* NCIMB 41125 were equally effective in metabolizing lactic acid. Lyophilized *M. elsdenii* resulted in less time below important pH thresholds, but no further benefit of daily administration was realized in this experiment.

Keywords: Lactipro *advance*, *Megasphaera elsdenii*, feedlot, accelerated step-up

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Dedication

I dedicate this thesis to my grandpa Bob Schecher. You made sure to pass your passion for cattle and love of the people of the cattle industry on to me. I would not be the person I am today without your encouragement to dream big and to chase my dreams relentlessly. You will forever be my inspiration and my guardian angel.

The Lord is my rock, and my fortress, and my deliverer; my God, my strength, in whom I take refuge; my shield, and the horn of my salvation, and my higher tower.

Psalm 18:2

Chapter 1 - Literature Review

History of *Megasphaera elsdenii*

Elsden and Lewis (1953) accidentally discovered a new bacterium while studying the ruminal microflora of sheep. This bacterium, referred to as organism LC in early literature, was an anaerobic, Gram-negative cocci that metabolized lactic acid, glucose, and fructose to yield H_2, CO_2, and volatile fatty acids (Elsden and Lewis, 1953). Production of pentanoate and heptanoate by organism LC sparked the interest of Elsden and Lewis (1953) because relatively few bacteria had been found to produce VFAs with carbon chains exceeding that of butyrate. When originally classifying organism LC, Elsden (1956) suggested this bacteria is closely related to the genres *Moraxella* and *Neisseria*. The former was overruled due to the fact that LC forms short chains of cocci, and the latter also did not fit LC because *Neisseria* is an obligate aerobe. The first classification attempt (Elsden et al., 1956) resulted in the recommendation to continue referring to organism LC as such, until adequate information was acquired to accurately classify the bacterium. Elsden (1956) also states "the possibility that it is a member of a new genus cannot be dismissed." Gutierrez et al. (1959) isolated "LC type organisms" from the rumens of cattle fed high grain diets. This paper proposed organism LC to be classified as *Peptostreptococcus elsdenii* due to morphology, production of VFAs as end products, and the ability to utilize organic acids as substrates. Gutierrez et al. (1959) also suggests that *P. elsdenii* could play an important role in rumal fermentation when high grain diets are fed due to the large number isolated from ruminal contents of animals fed this type of diet. In proposing this genus, Gutierrez et al. (1959) described organism LC as staining predominantly Gram negative, but in direct smears of ruminal samples variable results were observed within the LC-type cells. Rogosa (1971) reported organism LC is, in fact, Gram-negative (verified by Gram staining and electron

microscopy), whereas the genus *Peptostreptococcus* is characterized as Gram-positive bacteria. This led to the genus name *Peptostreptococcus* to be changed to *Megasphaera*. Broken down, *Megasphaera* means big sphere (mega: big, sphaera: sphere) and accurately describes morphology of the bacterium, as cells are large cocci with a diameter of 2 μm or larger. Today, organism LC is known as *Megasphaera elsdenii*. Although *M. elsdenii* was originally isolated from ruminants, this bacterium also has been isolated from the hindgut of many other mammals, including swine and humans (Marounek et al., 1989).

Probiotics and direct-fed microbials in ruminant diets

Probiotics and direct-fed microbials (DFM) are commonly used feed additives in diets of many livestock species, as well as in humans. Fuller (1989) defines the term probiotic as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance." This definition has been used to describe products that contain viable microbial cultures, enzyme preparations, culture extracts, or combinations of these components (Yoon and Stern 1995). Confusion due to the broad use of this definition caused the United States Food and Drug Administration (FDA) to require the use of the term direct-fed microbials, and narrow the definition to read "a source of viable, naturally occurring microorganisms" (Yoon and Stern 1995). Some of these feed additives are considered to have generally recognized as safe (GRAS) status. Under the section 201 of the Federal Food, Drug, and Cosmetic Act, feed additives are considered any substances that are intended for incorporation in feed or change characteristics of feed. Ingredients that fall under this definition are required to obtain premarket approval unless the product is eligible for a GRAS exemption (Rulis and Levitt, 2009). Generally recognized as safe status indicates that the additive is commonly considered safe, and

information proving so is available and agreed upon across scientists and food safety experts (Rulis and Levitt, 2009).

Fuller (1989) described characteristics of an effective probiotic for use in any animal, including humans. These characteristics include: providing benefit the host animal, not possessing pathogenic or toxic properties, containing viable cells in relatively large numbers, ability to thrive in the gastrointestinal tract environment, and stability of viable cultures through storage. Types of DFM that meet these criteria fall under 2 broad categories: fungal or bacterial.

Common fungal cultures used in ruminant diets are of the yeast species *Saccharomyces,* or the mold species *Aspergillus* (Seo et al., 2010). A review by Puniya et al. (2015) attributed benefits of fungal DFM to stimulation of both fibrolytic and lactate utilizing bacteria. By colonizing feed particles, ruminal fungi create more surface area for bacteria to proliferate and enzymes to attack, allowing increased fermentation of fibrous feedstuffs. Stimulation of lactate utilizing bacteria by fungal DFM allows for moderation of ruminal pH when high concentrate diets are fed (Seo et al., 2010). Waldrip and Martin (1993) reported improved lactic acid utilization by *M. elsdenii* when in the presence of a fermentation extract from *Aspergillus oryzae* cultures *in vitro*. Amino acid profile and B vitamin complex of the extract were believed to stimulate growth of *M. elsdenii*. Another proposed mode of action of fungal DFM is utilization of excess oxygen, which leads to a more ideal ruminal environment for anaerobic microorganisms (Puniya et al., 2015). Chaucheyras-Durand et al. (2008) summarized mode of action of fungal DFM well: increasing fermentation of low quality feedstuffs, providing micronutrients for ruminal bacteria, as well as maintaining optimal pH and anaerobosis which improves lactate and fiber digestion and rumen microbial establishment. As a result, increased microbial growth and microbial crude protein available to the ruminant, along with stabilization

of ruminal pH, are observed. These observations ultimately lead to improved productivity and overall health.

Modes of action of bacterial DFM can be ruminal or post-ruminal. Beneficial activities of bacterial DFM include competitive attachment, antibacterial effects, modulation of immune response, and changes in ruminal fermentation (Krehbiel et al., 2003; Puniya et al., 2015). Specific modes of action vary between types of DFM, dosage, and diet (Elghandour et al., 2015). Three major types of bacterial DFM have been researched (Seo et al., 2010): lactic acid producing bacteria (LAB), propionic acid producing bacteria (PAB), and lactate utilizing bacteria (LUB). Lactate producing bacteria are the predominant DFM bacteria commercially available to the beef and dairy industries (Puniya et al., 2015). These bacteria have been proposed to be active ruminally, aiding in stabilization of rumen pH (Seo et al., 2010). Yoon and Stern (1995) reported that continual supply of lactic acid helped microorganisms to adapt to lactic acid as well as stimulated LUB. Lactic acid bacteria also have been hypothesized to be beneficial in the large intestine and colon. A review by Seo et al. (2010) summarizes intestinal activity of LAB as including probiotic characteristics and competitive exclusion. Lactate producing bacteria, such as *Lactobacillus sp.*, compete for attachment sites on intestinal walls, preventing pathogenic bacteria (*E. coli, Salmonella sp.*, etc.) from adhering and proliferating in the gastrointestinal tract. Many pathogenic bacteria require this attachment to colonize and cause disease. End products of LAB fermentation of carbohydrates include lactic acid, VFAs, bacteriocins, and hydrogen peroxide, all of which interfere with cellular processes of pathogenic bacteria (Seo et al., 2010). This inhibition of pathogenic bacteria may result in reduced use of antibiotics, but should not be used to replace antibiotics as treatment for disease outbreaks (Walsh et al., 2012). Propionate producing bacteria are broadly classified under the genus

Propionibacterium. Propionate production from lactate is the major characteristic of PAB used to justify this group of bacteria as DFM (Krehbiel et al., 2003). The gluconeogenic nature of propionate allows propionibacteria supplementation to increase glucose production (Stein et al., 2006). Elevated levels of blood glucose result in increased energetic efficiency (Seo et al., 2010), increased milk production (Stein et al. 2006), and possible reduction in metabolic conditions such as ketosis (Stein et al., 2006). Lactate utilizing bacteria are characterized by their ability to metabolize lactic acid to produce VFAs. *Megasphaera elsdenii* is a major ruminal LUB and has been used as a DFM to modulate ruminal pH (Seo et al., 2010). As rapidly fermentable carbohydrates are added to ruminant diets, LUB are a viable option to protect ruminants from sudden, drastic decline in pH (Kung and Hession, 1995). Characteristics of the LUB *Megasphaera elsdenii* will be discussed in depth later in this review.

The digestive system of a pre-ruminant calf functions more similarly to that of a monogastric animal than that of an adult ruminant. Drackley (2008) described development of the rumen as occurring in 3 stages: pre-ruminant, transition, and ruminant phases. The pre-ruminant phase lasts the first 2 to 3 weeks of life and is characterized by primary consumption of liquid nutrient sources (milk or milk replacer). Suckling triggers closure of the esophageal groove, allowing liquids to avoid the reticulorumen and to pass directly to the omasum and abomasum to begin digestion. Large amounts of nutrients reaching the intestines, accompanied by developing intestinal flora, open the door for colonization by pathogenic bacteria, resulting in disease (Uyeno et al., 2015). At birth, calves have sterile digestive systems that must be colonized (Uyeno et al., 2015). Processes of colonization differ among production systems. Calves born into social production systems, with unlimited access to the cow and other animals, usually will achieve complete ruminal and intestinal microflora through contact with other

animals. In commercial dairy production systems, calves generally are allowed limited access to the cow and are reared in non-social confinement, which can lead to deficiencies in gut microflora. Probiotics may be an option to aid in colonization of the GI tract, thus protecting calves from pathogenic bacteria in this type of production system (Fuller, 1989). Common bacterial DFM used in pre-ruminant calves are *Lactobacillus sp*. (Seo et al., 2010) which have been reported to decrease fecal shedding of pathogenic bacteria such as *E. coli* (Elam et al., 2003), thus decreasing incidence of diarrhea and scours (Abe et al., 1995; Abu-Taraboush et al., 1996). Antibiotics have been included in dairy calf diets to minimize susceptibility to pathogenic bacteria and disease. Martínez-Vaz et al. (2014) noted that antibiotic replacement of antibiotics by DFM in calves is possible to protect against pathogens, but should not replace antibiotics for disease treatment (Walsh et al., 2012).

As calves progress to the transition phase, solid feed is consumed in the form of grazing, or creep/starter feeds (Drackley, 2008). Ingestion of solid feeds diminishes the suckling reflex, and feedstuffs are introduced to the ruminal flora. This change in diet triggers a shift in metabolism, as microbes adapt to fermentation of carbohydrates to VFAs (Uyeno et al., 2015). Volatile fatty acids, namely butyrate, and to some extent propionate, stimulate physiological changes such as GIT epithelium development and papillary growth. Increased musculature, vasculature, and total volume of the rumen also are observed in the transition phase (Drackley, 2008). Calves with an intestinal imbalance created by feeding spray dried whey powder, supplemented with *Lactobacillus sp.* and *Pediococcus sp.* began eating starter feed, and thus experiencing ruminal development, at an earlier age (Frizzo et al., 2010). Bacterial DFM also have been used as a means to improve ADG and feed efficiency in pre-weaned calves (Abe et al., 1995; Malik and Bandla, 2010).

As milk is removed from the animal's diet during weaning, fermentation of carbohydrates, absorption of VFAs, and microbial crude protein become increasingly important in the digestion of feedstuffs (Drackley, 2008). The microbial, metabolic, and physiological changes mentioned previously in the transition phase are essential to maintain healthy digestion in adult ruminants. High levels of stress due to separation from the cow, vaccination, new location, and diet change characterize weaning. Stress can impact performance and health of cattle due to altered microbial populations and decreased immunity (Krehbiel et al., 2003). Combined results from multiple studies in the 1980s observed increased ADG, DMI, and feed efficiency, and decreased morbidity among calves fed various bacterial DFM products (Krehbiel et al., 2003). Krehbiel et al. (2001) and Kiesling and Lofgreen (1981) did not observe improvements in calf performance from DFM inoculation, but fewer calves were retreated with antimicrobials when inoculated with DFM (Krehbiel et al., 2001).

Substrate utilization by *Megasphaera elsdenii*

Substrate preference

Megasphaera elsdenii is a fermentative bacterium able to utilize a wide range of carbohydrates and organic acids (Marounek et al., 1989). Elsden and Lewis (1953) originally determined organism LC could ferment D,L-lactate and several sugars, including glucose, fructose, maltose, mannitol, and sorbitol. Consistent growth has been reported when *M. elsdenii* is grown on glucose, fructose, and DL-lactate (Elsden and Lewis, 1953; Russell and Baldwin, 1979; Hino and Kuroda, 1993), but fermentation is more variable with other carbohydrate substrates (Russell and Baldwin, 1979). Russell and Baldwin (1978) compared substrate utilization of various ruminal microorganisms and report respectable growth rates of *M. elsdenii*

grown on glucose, maltose, and lactate, with no inhibition by other substrates. Sucrose utilization, on the other hand, was inhibited by the presence of glucose and maltose. A recent study by (Mobiglia et al., 2017) evaluated *in vitro* fermentation of 14 different substrates by *M. elsdenii* NCIMB 41125. Fructose yielded the greatest total growth, with glucose, lactate, and maltose to follow. Marginal growth was detected when fructo-oligosaccaride, raffinose, xylose, sucrose, and soy protein were supplied as carbon sources. Succinate was found to be inhibitory to growth when offered as the primary carbon source.

In the rumen ecosystem, multiple substrates are available to microbes simultaneously. The ability to utilize a wide range of substrates makes *M. elsdenii* a suitable bacterium to inhabit the rumen, but does this organism prefer to use a specific substrate? Marounek et al. (1989) grew *M. elsdenii* in media containing 20 mM sodium lactate with 10 or 20 mM glucose and found in both cases glucose utilization began as lactate concentration was depleted. This study suggested a preference for lactate and diauxic growth when glucose and lactate are offered simultaneously. Hino and Kuroda (1993) conducted a similar study, providing 20 mM sodium lactate and 4 mM glucose in growth medium. This experiment also suggested a preference for lactate over glucose, with initiation of glucose utilization occurring only when lactate concentration was between 1 and 2 mM. Because glucose catabolism starts prior to exhaustion of lactate, growth slows as cells transition to glucose utilization, but there is no cessation of growth characteristic of diauxic growth. Lactate was also added to cultures actively fermenting glucose (Hino and Kuroda, 1993). This experiment offered further evidence of lactate preference over glucose, as cultures transitioned to lactate utilization while suppressing glucose catabolism until lactate concentration was again reduced to 2 mM. Russell and Baldwin (1978) reported a preference by *M. elsdenii* for glucose and maltose over sucrose. Both substrates caused active sucrose utilization to cease until

concentrations of the inhibitors were very low. This was attributed to end product inhibition regulatory mechanisms.

Although *M. elsdenii* is well known for its major role in catabolism lactic acid in the rumen, it also has the ability to catabolize amino acids (Rychlik et al., 2002). Deamination of amino acids by microorganisms is an energetically expensive process that generally occurs for one of two reasons: ATP or ammonia production. Although an inefficient process, deamination provides important growth factors such as branched-chain volatile fatty acids (BCVFAs) and ammonia required by cellulolytic bacteria. Branched chain volatile fatty acids are produced from catabolism of branched-chain amino acids (BCAAs). Lewis and Elsden (1955) observed fermentation of threonine, serine, cysteine, and acrylic acid to ammonia, hydrogen, carbon dioxide, and VFAs. Wallace (1986) suggested energy from amino acid catabolism is utilized minimally for growth, but may contribute to the maintenance energy of *M. elsdenii*, as BCVFA production is increased during the stationary phase of growth as compared to the exponential phase. Physiological concentrations of ruminal carbohydrates are generally high immediately after feeding and are nearly depleted within a few hours (Allison, 1978). With BCVFA production at its greatest with low levels of available carbohydrates, amino acid catabolism may be an important process in maintaining populations between feedings.

Transport and pathways

Transport

The initial process for metabolism of nutrients by ruminal bacteria is transport into the cell. Six transport mechanisms for carbohydrates exist in microorganisms: passive diffusion, facilitated diffusion, shock sensitive systems, proton symport, sodium symport, and the phosphoenolpyruvate-dependent phosphotransferase system (PEP-PTS, Martin, 1994). Passive

and facilitated diffusion transport solutes down a concentration gradient either randomly (passive), or with the aid of a carrier protein (facilitated). The remaining transport mechanisms are referred to as active transport, requiring energy to move solutes against concentration gradients. Shock sensitive systems also are referred to as primary active transport. This process involves periplasmic sugar-binding proteins and specific membrane porins and proteins, and is directly coupled with chemical energy such as ATP, phosphoenolpyruvate (PEP), or acetyl-phosphate (Martin, 1994). Proton and sodium symport are the co-transport of a solute coupled with an ion (i.e., H^+ or Na^+). Substrate phosphorylation, an ATP requiring process mediated by kinase enzyme activity, is required to instigate glycolysis. The last transport mechanism is PEP-PTS. This system phosphorylates the substrate during intracellular transport via the donation of a phosphate group by phosphoenolpyruvate. In energy-limited environments such as the rumen, PEP-PTS transport mechanism allows bacteria to conserve ATP (Martin, 1994). A schematic of these transport systems is shown in Figure 1.1.

Glucose and lactate are major substrates that are utilized by *M. elsdenii*, and must be transported into the cell. Evidence for presence of PEP-PTS has been reported for glucose (Martin and Russell 1986; Chen and Russell 1989; Martin 1994; Martin and Wani 2000) as well as fructose (Dills et al., 1981; Martin, 1994). Dills, Lee, and Milton (1981) reported PEP-PTS is inducible for both glucose and fructose due to suggested regulation mechanisms evolved in limiting accumulation of sugars as the cells approach the stationary phase. Although, in 2000, Martin and Wani found PEP-dependent phosphorylation of glucose was constitutive, as activity was detected in cultures grown on glucose, maltose, and lactate media.

Lactate transportation has been attributed to proton motive force (Waldrip and Martin, 1993). Studies have reported stimulation of lactate catabolism in environments with pH between

4.0 and 6.0 (Therion et al., 1982; Waldrip and Martin, 1993). This, combined with Waldrip and Martin's (1993) discovery that uncoupling agents reduce lactate uptake by up to 61%, supports the hypothesis that proton motive force is the mechanism underlying lactate uptake. Waldrip and Martin (1993) also demonstrated that neither decreasing cation concentration (sodium or potassium) nor interfering with cation gradients inhibited lactate uptake by *M. elsdenii*. The same study also suggested that both D- and L-lactate utilize the same membrane carrier.

Pathways

Different metabolic pathways are used to ferment glucose and lactate in *M. elsdenii*, yielding different end products result from fermentation of the two substrates (Marounek et al., 1989). Glucose is metabolized principally to acetate and butyrate (Elsden et al., 1956; Forsberg, 1978; Prabhu et al., 2012; Weimer and Moen, 2013). Major end products of L-lactate fermentation are acetate and butyrate, while D-lactate yields propionate (Hino and Kuroda, 1993). A schematic of metabolism of both glucose and lactate can be found in Figure 1.2.

Romano et al. (1970, 1979) reported the PEP-PTS transport mechanism is found only in bacteria that utilize the Embden-Meyerhof-Parnas (EMP) pathway. Linkage of these two processes conserves ATP, which proves vital to microorganisms in anaerobic environments such as the rumen (Romano et al., 1970; Romano et al., 1979; Martin and Russell, 1986). Phosphorylation of the sugar in transit yields an intermediate product able to enter directly into the EMP pathway (Romano et al., 1979), thus avoiding energy-using conversion steps (Roseman, 1969). End products of EMP from glucose are 2 PEP, which are dephosphorylated by either pyruvate kinase to generate ATP, or phosphotransferase to serve as energy in importing another glucose molecule (Roseman, 1969). In both cases, the dephosphorylation of PEP generates pyruvate, which is metabolized to VFAs.

Pyruvate also can be formed as an intermediate product in lactate fermentation. Nicotinamide adenosine dinucleotide-independent lactate dehydrogenase (iD-LDH) is an enzyme that catalyzes a reversible reaction that converts D-lactate to pyruvate, and vice versa, under certain circumstances. This reaction normally favors pyruvate production unless the pyruvate:D-lactate ratio increases enough to drive the reverse reaction to occur. To achieve this ratio, D-lactate must be converted to L-lactate, a process that can be driven by production of propionate via the acrylate pathway. Lactate racemase (LR) is the enzyme that must be present to convert L-lactate to D-lactate and vice versa (Hino and Kuroda, 1993). Hino and Kuroda (1993) reported that in *M. elsdenii* cultures grown in DL-lactate, similar levels of fermentation of D- and L-lactate occur, indicating that LR is equally active in conversion from D-lactate to L-lactate as from L-lactate to D-lactate. This reversible nature allows oxidation of D-lactate to pyruvate, with subsequent metabolism to acetate and butyrate, and L-lactate metabolism to propionate to occur simultaneously (Prabhu et al., 2012).

Lack of propionate production is one major difference in the products of fermentation for glucose and lactate. As previously mentioned, L-lactate is metabolized to propionate via the acrylate pathway in *M. elsdenii*. This is a defining characteristic of this microorganism, as few bacteria have been identified to use this pathway, which requires action of LR as well as iD-LDH enzymes to maintain adequate L-lactate. Interestingly, iD-LDH is present in *M. elsddenii* cells grown in glucose media, but activity is reduced by half compared to cells grown in lactate. Lactate racemase is not produced by *M. elsdenii* cells grown in glucose *in vitro* systems (Hino and Kuroda, 1993; Hino et al., 1994). Hino et al. (1994) conducted a study that evaluated activity of iD-LDH and LR when a substrate shift occurs. Activity of both enzymes increased rapidly with lactate addition to an actively growing glucose culture and decreased to basal (iD-LDH) or

non-existent (LR) levels as lactate concentration diminished. These results demonstrate the inducible nature of LR and constitutive characteristic of iD-LDH in *M. elsdenii*. Glucose grown cells do not have the capability to produce propionate because both of these enzymes are required for propionate production to occur. In lactate grown cells, propionate is the major end product from the fermentation of L-lactate. Lactate induces activity of LR and increases activity of iD-LDH, allowing the acrylate pathway to function. A detailed schematic of the acrylate pathway can be found in Figure 1.3. A study conducted by Prabhu et al. (2012) reported that inclusion of acrylate in a 35 mM lactate medium increased propionate production, but *M. elsdenii* did not grow with acrylate as the sole carbon source.

Pyruvate generated as an intermediate of transport and metabolism of glucose and D-lactate is converted to acetyl CoA by pyruvate oxidoreductase. Unlike other bacteria that utilize pyruvate formate lyase, this reaction does not result in formate production. Acetyl CoA is then metabolized to yield acetate and butyrate. In a study conducted by Marounek et al. (1989), increasing glucose in media resulted in increased proportion of butyrate production. Cells grown with acrylate included in lactate media produced a lower proportion of butyrate in a study conducted by Prabhu et al. (2012). In the same study, increased proportions of butyrate relative to acetate were observed when higher proportions of D-lactate or glucose are metabolized compared to L-lactate. This may be due to balanced redox potential from the reduced number of electrons generated. To further support this conclusion, butyrate production allows cells to consume NADH without utilizing ATP.

Another role of *M. elsdenii* is the production of the BCVFAs isobutyrate, isovalerate, and 3-methylbutyrate from the BCAAs valine, leucine, and isoleucine, respectively (Allison, 1978;

Wallace, 1986; Rychlik et al., 2002). This is achieved by a series of reactions: transamination, decarboxylation, phosphorylation, reduction, and dephosphorylation.

Fates of end products

Although acetate and propionate are produced by *M. elsdenii* in similar proportion, acetate is the predominant VFA produced in the rumen as a whole. Acetate may be absorbed by the rumen epithelium and metabolized in the liver or by body tissues for energy (Ballard, 1972) or may serve as a precursor for lipid synthesis in liver and adipose tissues (Hanson and Ballard, 1967). A study conducted by Annison and Lindsay (1961) reported increasing concentrations of acetate from the rumen contents to portal blood and the highest concentration in jugular blood, making it apparent body tissues utilize acetate more so than splanchnic tissues. Some acetate may also be used by other ruminal microorganisms to produce butyrate (Hino, Miyazaki, & Kuroda, 1991) or methane (Nagaraja, 2016). Bergman et al. (1966) reported that minimal glucose is absorbed from the portal drained viscera of ruminants. This observation is due to microbial fermentation of glucose to VFAs. Propionate is the only VFA produced that is considered gluconeogenic, and the vast majority is absorbed by hepatic tissue for use in gluconeogenesis. Annison and Lindsay (1961) supports this with their finding that propionic acid concentration is decreased in portal blood as compared to ruminal fluid and is nonexistent in jugular blood of sheep fed 3 different diets.

A majority of butyrate is metabolized by the ruminal epithelium. Annison and Lindsay (1961) reported minimal butyric acid in portal blood or jugular blood, while molar proportions in the rumen are relatively elevated. Butyrate is known for its role in development of epithelial tissues in the GI tract, including the rumen (Sakata and Yajima, 1984).

Although branched-chain VFAs are produced in small molar proportions in the rumen, they are very important growth factors for cellulolytic microorganisms (Wallace, 1986; Rychlik et al., 2002). A review by Allison (1969) reported synthesis of branched-chain cellular components including amino acids, fatty acids, and aldehydes, among others all are products of ruminal microbial utilization of BCVFAs.

Ruminal pH and acidosis

Metabolic disorders are an important cause of morbidity and mortality among feedlot cattle, ranking second only to respiratory diseases (Smith, 1998; Loneragan et al., 2001). Ruminal acidosis has been identified as a major metabolic disorder in beef cattle that negatively impacts feedlot performance (Nagaraja and Lechtenberg, 2007; Nagaraja and Titgemeyer, 2007). This fermentative disorder can be described as a decrease in ruminal pH due to production of organic acids in excess of capability of ruminal buffering capability and epithelial absorption (Hernández et al., 2014), and generally is measured by ruminal fluid pH (Plaizier et al., 2008). The complexity of ruminal acidosis etiology makes economic losses to the feedlot industry difficult to quantify: direct losses manifest as decreased ADG and poorer feed efficiency (Castillo-Lopez et al., 2014), while additional losses can be attributed to subsequent diseases such as rumenitis, bloat, laminitis, and liver abscesses (Millen, 2016).

Ruminal acidosis can be classified into two categories: sub acute ruminal acidosis (SARA) or acute ruminal acidosis. Nagaraja & Lechtenberg (2007) described ruminal characteristics of these classifications well. Sub acute ruminal acidosis is defined by the pH range of 5.0 to 5.6, with the increase in organic acids attributed to elevated concentrations of VFA (150 to 225 mM). An interesting shift in organic acid proportions occurs in the transition to

acute acidosis, in that total VFA concentrations decrease to lower than normal concentrations (< 100 mM) and lactic acid increases to 50 to 120 mM (normal 0 to 5 mM). Lactic acid is a much stronger acid than VFAs, with a pKa of 3.9 as compared to 4.9, respectively. This shift to a stronger acid causes a decrease in pH to less than 5.0.

Ruminal microorganisms vary in their degree of acid tolerance, causing changes in pH to affect different groups of microorganisms differently. Dramatic reductions in ciliated protozoa populations are observed in SARA (Goad et al., 1998), which does not come as a surprise, as one of the primary methods of experimental defaunation is decreasing ruminal pH (Nagaraja & Titgemeyer, 2007). Declining populations of cellulolytic and fibrolytic bacteria are also observed as pH drops below 6 (Plaizier et al., 2008). Decreases in fiber fermenting bacteria result in decreased fiber fermentation and increased proportion of concentrate degradation. Kung & Hession (1995) reported that *M. elsdenii* remains competitive in the rumen until pH drops below 5.4.

An interesting interaction between bacterial species plays a large role in the etiology of acute acidosis. *Streptococcus bovis* is a LAB that thrives in conditions where rapidly fermentable carbohydrates are available in excess inunadapted cattle. This microbe's major end product is lactic acid, and rapid growth of *S. bovis* leads to decreases in ruminal pH to levels that are inhibitory to a vast majority of ruminal microorganisms, including *S. bovis* itself and lactate utilizing bacteria. Impressive acid tolerance of *Lactobacillus* spp. allows these bacteria to continue to grow and become dominant in the rumen at pH below 5.6. The major end product of *Lactobacillus* also is lactic acid. Inhibition of LUB, compounded by stimulation of LAB, leads to an environment in which lactic acid accumulates (Nagaraja & Titgemeyer, 2007). Cattle that are adapted to high-grain diets are able to metabolize and prevent acidosis due to sufficient

concentrations of LUB. *M. elsdenii's* ability to metabolize 60 to 80% or ruminal lactic acid makes it well equipped to fight acidosis (Counotte et al., 1981).

Cattle are at greatest risk of lactic acidosis in 2 phases of the finishing period: step-up and late finishing (Nagaraja and Lechtenberg, 2007). Accumulation of lactic acid occurs when production exceeds metabolism and absorption. In unadapted cattle, populations of *M. elsdenii* and other LUB are underdeveloped and lack capacity to metabolize large quantities of lactic acid. If care is not taken to ensure microorganisms of the rumen are adapted to increased fermentable carbohydrates, lactic acid accumulation will occur (Nagaraja & Titgemeyer, 2007). As cattle grow, DMI increases, especially in the late finishing phase. Increased consumption of high concentrate diets elevates risk of SARA and acute acidosis. Multiple factors can contribute to the accumulation of organic acids: DMI fluctuations, extreme weather events (heat, cold, precipitation, etc.), injury or disease, poor bunk management, and palatability, among other factors (Millen, 2016; Nagaraja & Lechtenberg, 2007).

Symptoms of acidosis vary depending on classification of the disorder. Sub acute ruminal acidosis is very difficult to diagnose, as few symptoms can be observed (Nagaraja and Lechtenberg, 2007). Anorexia, lethargy, diarrhea, and increased respiratory rates can be observed and should lead to investigation of the animal. These symptoms also are characteristic of many other metabolic disorders and diseases. Symptoms of lactic acidosis, though similar to SARA, are intensified and can be detected more readily. Cattle are uncoordinated, followed by lethargy, loss of appetite, and decreased ruminal motility. Dehydration often is observed 24 to 48 h after onset of lactic acidosis, and feces progress from soupy, to watery, and eventually to foamy (Nagaraja and Lechtenberg, 2007). In cases of severe peracute acidosis, animals can die within 6 to 16 h. Animals that recover from acute acidosis often have inflamed ruminal papillae that are

keratinized, which decreases absorptive capacity of the rumen (Plaizier et al., 2008). Other metabolic disorders and diseases, including rumenitis, laminitis, bloat, and liver abscesses, can arise from damage to the rumen (Millen, 2016).

Effective treatments are very limited for acidosis, therefore prevention is key. Since causes for this disorder are brought on by an overload of fermentable carbohydrates, nutritional management is crucial for prevention of acidosis. Following bunk management protocols, utilizing transition or step-up regimens, and formulating diets suitable to the needs of cattle all are precautionary steps for minimizing risk of acidosis. Increasing roughage levels can improve performance as well as ruminal health and epithelial integrity in cattle that are battling acidosis. Knowledge of how amount, type of grain, and methods of cereal grain processing affect starch availability can help in management of acidosis (Nagaraja and Lechtenberg, 2007; Millen, 2016). Alternative energy sources, such as byproduct feedstuffs, provide quality nutrients with the absence of starch. Distillers grains, corn gluten feed, and many other byproducts have been suggested to control acidosis by replacing a portion of cereal grains in feedlot rations, reducing starch intake (Nagaraja & Lechtenberg, 2007). Ionophores, nonionophore antibiotics, buffers, organic acids, and probiotics are feed additives available to producers that aid in reduction of acidosis in different ways.

Transitioning cattle to high concentrate diets

Generally, newly received cattle do not eat well immediately upon arrival. Receiving cattle on a familiar, palatable roughage source, such as long-grass or alfalfa hay, is common practice because many cattle are sourced from pasture or forage based management programs (Preston, 2007). Adaptation to finishing diets from these forage based diets is a crucial time in the life of a feedlot animal. Brown et al. (2014) define adaptation as the time an animal can be

fed a diet at a rate that previously would have caused metabolic disturbances without negative consequences. The abrupt transition to high concentrate diets can lead to multiple metabolic disorders, including acidosis, with long-term consequences or potential lethality. The major goal of adaptation is smooth transition to the finishing diet (Duff, 2007).

A survey of 29 consulting feedlot nutritionists conducted by Vasconcelos and Galyean (2007) indicated the majority of nutritionists use a step-wise adaptation program with incremental increases in concentrate utilizing 2 to 3 intermediate diets. Another common transition program revealed by this survey is ration blending, where 2 diets are fed in separate feedings to progress from low to high concentrate intake. For either system, average time until cattle consume finishing diets is 21 d. The majority of nutritionists surveyed also begin step-up programs at 40 to 45% roughage and progress toward final diets that contain between 5 and 10% roughage.

Ad libitum bunk management offers cattle unlimited access to feed. Brown et al. (2014) reports a reduction in ADG and feed efficiency in the first 21 d after cattle transition from *ad libitum* forage intake to *ad libitum* consumption of concentrate based diets. This can be attributed to bio-behavioral control systems (Preston, 2007). The rumen serves a role in regulation of intake, mostly by tension receptors detecting gut fill. Decreasing bulky roughage in a diet also decreases an animal's ability to eat to achieve gut fill, instead it must depend on metabolic satiety signals to control feed intake. Overconsumption of readily fermentable carbohydrates occurs when this regulatory system is disturbed by an abrupt diet change, and leads to the accumulation of fermentation end products, including lactic acid. As mentioned earlier, build up of lactic acid causes acidosis, and a subsequent decrease in DMI. In order to use *ad libitum* bunk management systems in step up programs, incremental step-wise transition programs are

recommended (Choat et al., 2002; Duff, 2007; Preston, 2007). Pronounced variability in feed intake can be observed not only with *ad libitum* step-up programs, but also throughout the feeding period (Choat et al., 2002; Duff, 2007; Preston, 2007).

Limit feeding or restricted intake is a common alternative to *ad libitum* step-up programs. This adaptation strategy places cattle directly on a finishing diet but limits intake by a percentage of body weight to prevent over consumption (Choat et al. 2002; Preston 2007; Duff 2007; Brown et al. 2014). Duff (2007) offers multiple reasons for utilizing this feeding system: decreased roughage use, decreased intake variability, simplified bunk management, decreased manure output, and improved feed efficiency. Multiple sources report a decrease in DMI and ADG but improved feed efficiency in the first 28 d of consuming high concentrate diets (Choat et al. 2002; Preston 2007; Duff 2007; Brown et al. 2014). This can be expected, as feed intake and energy are limited by design. By restricting intake, Choat et al. (2002) showed variability of DMI is managed much more efficiently than in *ad libitum* systems.

Application of *Megasphaera elsdenii*

Adaptation of ruminal microbiota to high concentrate feedlot diets is a very important process that supports the common recommendation of a 21-d step-up program. *Megasphaera elsdenii* is the predominant LUB in the rumen, as it is believed to metabolize 60 to 80% of lactic acid produced (Counotte et al., 1981). In a review, Meissner et al. (2010) reported that *M. elsdenii* NCIMB 41125 maintained higher pH, metabolized more lactic acid, and decreased time spent under both pH 6.0 and 5.0 in continuous culture better than a control culture containing no *M. elsdenii* and the type strain of *M. elsdenii* (ATCC 25940).

Providing LUB to the rumen by drenching cattle with *M. elsdenii* directly before transitioning to concentrate diets allows for implementation of accelerated step-up programs

without negative consequences (Miller, 2013; Henning et al., 2010; Drouillard, 2012; Leeuw et al., 2009). Variable effects on DMI and ADG have been observed during the first 28 to 30 d on feedlot rations when *M. elsdenii* is used. Miller et al. (2012) reported decreases in DMI for cattle on accelerated step-up programs (from 0 to 15 d) , although this may be due to differences in diet roughage and resulting gut fill. Even when accelerated adaptation periods were used, there were no differences in DMI, ADG, or feed efficiency, suggesting the decreased intake in the first 30 d was not due to lactic acidosis. Increased marbling scores were observed for treatments that were stepped up onto finishing diets earlier. Concentrate diets encourage intramuscular fat production more than forage-based diets, so this finding may be due to increased time on finishing diets. Contrary to Miller's findings, improvements in feedlot performance have been reported in the first 30 d during and after dietary transition (Henning et al. 2010; Drouillard et al. 2012; Leeuw et al. 2009). Henning et al (2010) reports a 21% increase in DMI for *M. elsdenii* drenched cattle over the control cattle with either abrupt or gradual transition to concentrate diets. This study also associates lower ruminal lactic acid concentrations with elevated *M. elsdenii* counts. Morbidity peaks in feedlots when cattle are freshly received. Leeuw et al. (2009) reported a decrease in animals treated for digestive disturbances (bloat and diarrhea) when cattle were drenched with *M. elsdenii* at initial processing.

Potential of freeze-drying

Lactipro *advance* (commercially available *M. elsdenii* NCIMB 41125) is effective as a fresh oral drench, allowing utilization of accelerated step-up programs. Today, this product has 3 major setbacks: short 14-d shelf life, large dose size (50 mL for feedlot cattle), and difficult administration, as cattle must be brought to a chute to receive the probiotic. Developing a preserved version of *M. elsdenii* would address all three of these issues. Many drying methods

are used across the food, pharmaceutical, and microbiological industries. For example, spray drying is a cost-effective, high throughput method that forms droplets and dries material simultaneously by forcing slurry through an atomizing nozzle into a drying chamber with high temperatures (>80°C; Peighambardoust et al., 2011). This method uses temperatures and air exposure that is lethal to *M. elsdenii*. Freeze-drying is a common method used to preserve bacteria; unfortunately, there is no universal method for freeze-drying microbes (Morgan et al., 2006). Varying characteristics of microorganisms and environments such as specie, strain, growth medium, growth phase, pH, composition of the freezing medium, and storage conditions can influence drying procedures (Hubalek, 2003) and dramatically affect cell recovery. A review by Morgan et al. (2006) summarized general steps in majority of drying procedures: organisms must be cultured, mixed with a drying protectant, frozen, dried, stored, rehydrated, and recovered for successful preservation to occur. Growth of bacteria occurs in 4 stages: 1) lag phase to adapt to the environment, 2) log phase where exponential growth occurs, 3) stationary phase where doubling ceases generally due to depletion of some nutrient, and 4) death phase. Ideal cell concentration and phase to stop growth and start the freeze-drying process is heavily dependent on the microorganism of interest (Morgan et al., 2006). Palmfeldt et al. (2003) reported that optimal cell concentration of *Pseudomonal chlororaphis* to be between 1×10^9 and 1×10^{10} CFU/mL, and that falling outside the range of 5×10^8 to 1×10^{10} CFU/mL was detrimental to the survival of the bacterium. In addition to optimizing cell concentration, stressors can be added to trigger stress responses to enhance survival. Carbon starvation, acid stress, heat or cold treatment, and osmotic stress are all adverse conditions to stress the microorganisms in attempt to increase desiccation tolerance (Morgan et al., 2006; Palmfeldt et al., 2003). In the case of Palmfeldt et al. (2003), only carbon starvation resulted in improved cell recoveries of *P.*

chlororaphis after freeze-drying. Palmfeldt and Hahn-Hagerdal (2000) on the other hand, reported a 28% improvement in cell recoveries of *Lactobacillus reuteri* when reducing pH was decreased from 6.0 to 5.0.

Mixing cultures with a cryoprotectant is the next general step. A cryoprotectant is a substance that protects tissues from damage during freezing, and can be categorized as eutectic crystalizing salts, or amorphous glass forming (Morgan et al., 2006). Salt crystal forming cryoprotectants form salt crystals in the freezing process and as water leaves the solution salts crystalize around the cells in a highly concentrated structure. Waste products retained in the crystals when combined with the high concentration of salt, make an environment that is conducive to cell damage. Amorphous glass forming cryoprotectants form a "supersaturated thermodynamically unstable liquid with very high viscosity" (Morgan et al., 2006). The viscosity of these solutions allows minimal molecular movement of cells during the drying process. An advantage of amorphous glass forming cryoprotectants is their ability to draw waste products away from cells before freeze-drying (Morgan et al., 2006). Many cryoprotectants have been successfully used in the freeze-drying of bacteria, but few yield satisfactory results on a broad range of microorganisms. A few broadly used cryoprotectants are: glycerol, serum, skimmed milk, yeast extract, peptone, glucose, sucrose, sorbitol, and trehalose (Hubalek, 2003). The optimal cryoprotectant for *P. chlororaphis* was 100 g/L sucrose, resulting in a 27% improvement in cell recovery in comparison to 100 g/L trehalose (Palmfeldt et al., 2003). Concentration of cryoprotectant is as important as selecting the optimal concentration or mixture; Palmfeldt et al. (2003) reported increasing cell survival between 50 and 130 g/L, but at 300 g/L survival of *P. chlororaphis* was decreased drastically.

After cultures are mixed with cryoprotectants, samples must be frozen, either by snap freezing with liquid nitrogen, or by being directly placed in the freeze-dryer. Snap freezing is commonly used, this process allows samples to freeze quickly, avoiding development of large ice crystals that can harm cells during the freeze-drying process (Morgan et al., 2006). If samples are placed directly into the freeze-drying machine, an annealing step should be added to the freeze-drying cycle. Annealing builds ice crystals with channels for moisture to escape via controlled rising and falling temperatures (Morgan et al., 2006). Once samples are frozen the two-step drying process begins. Primary drying vaporizes ice crystals via sublimation at low temperatures. This ensures no ice crystals melt, which would cause the pellet to collapse. Sublimation is transitioning from solid form to vapor form and requires pressure inside the chamber to be below the vapor pressure of the ice within the sample (Figure 1.4; Morgan et al., 2006). Secondary drying commences when all unbound ice has vaporized, and residual moisture bound to the sample is then removed at a higher temperature (Morgan et al., 2006). Rehydration and verification that cells survived the drying process is then carried out.

Interest in developing stable forms of bacteria and viruses for human vaccines has been expressed. Like Lactipro *advance*, many vaccines are stored in liquid forms that require refrigeration for stability. Preservation by lyophilization would allow extended storage of microorganisms and vaccines. Garmise et al. (2007) addressed this obstacle for whole inactivated influenza virus vaccinations. An improved method of administration was also developed in the process: intranasal administration. This version was equally effective as an intramuscular injection in mice, is heat stable without refrigeration, and would require minimal training for those who administer the vaccine. Several species of bacteria have also been successfully freeze-dried for many different uses. One example is the successful freeze-drying of 3 different species

of *Lactobacillus* in order to produce a stable pharmaceutical option for probiotic prevention of metritis (Otero, Espeche, and Nader-Macias, 2007). These successes offer encouragement for similar approaches with *M. elsdenii*.

Summary

M. elsdenii is a very important ruminal microorganism that metabolizes lactic acid and carbohydrates into VFAs. The affinity of this microbe for lactic acid allows it to play a major role in mitigation of acidosis, and accelerated step-up programs have been proven to be possible due to this characteristic. Previous success of freeze-drying both viruses and bacteria for medical purposes for humans and animals sparks interest in this as a preservation method for *M. elsdenii*. Development of a freeze-dried version of Lactipro *advance* would extend shelf life, and could potentially be administered in feed to improve ease of administration and offer protection against metabolic insults later in the feeding period.

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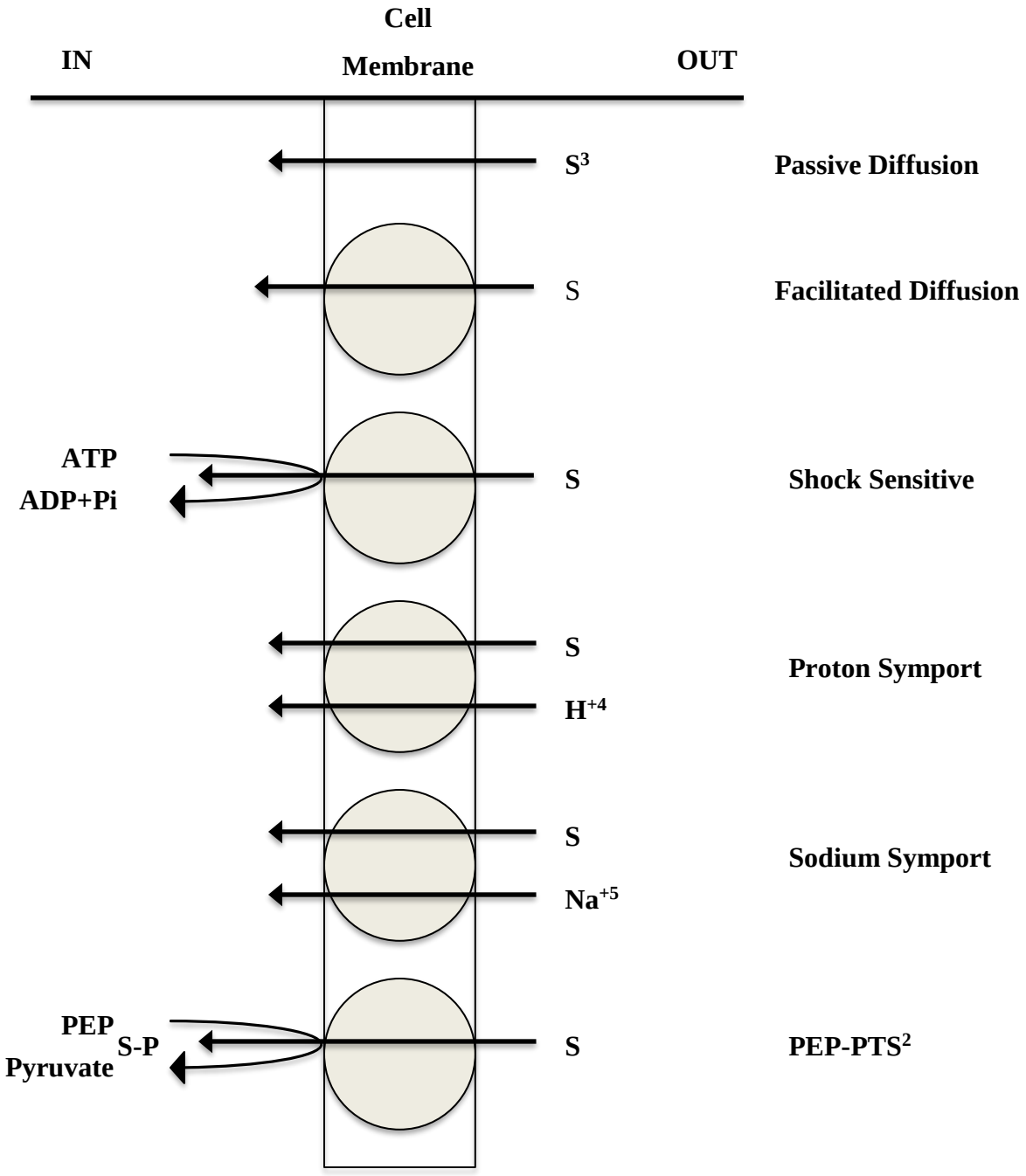
Figure 1.1 Summary of transport mechanisms identified in bacteria



[1] Adapted from Martin (1994)

[2] PEP-PTS = phosphoenylpyruvate phosphotransferase system

[3] S = substrate

[4] H^+ = proton

[5] Na^+ = sodium ion

Figure 1.2 Schematic of production of major VFAs from metabolism of glucose and lactate

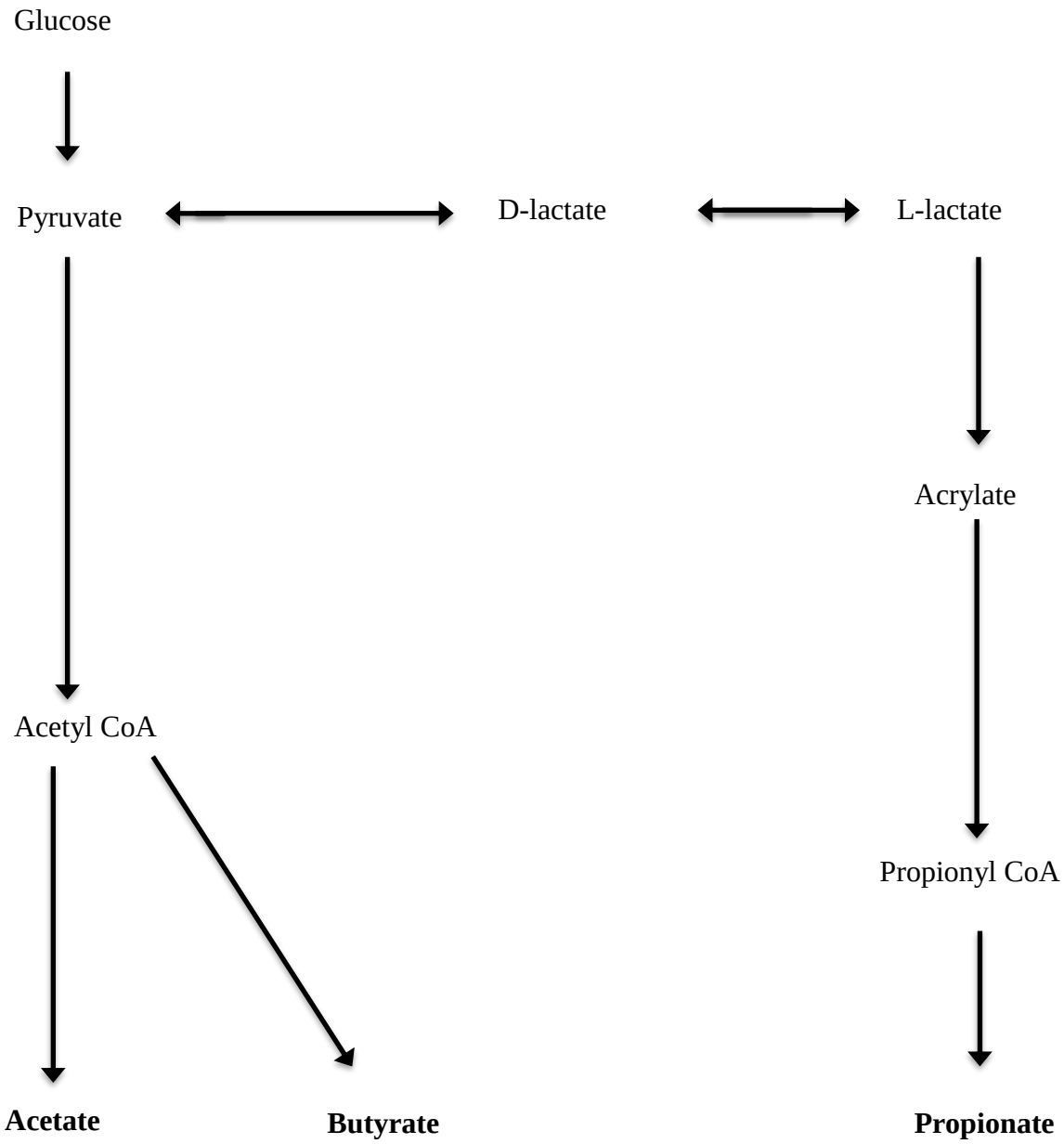
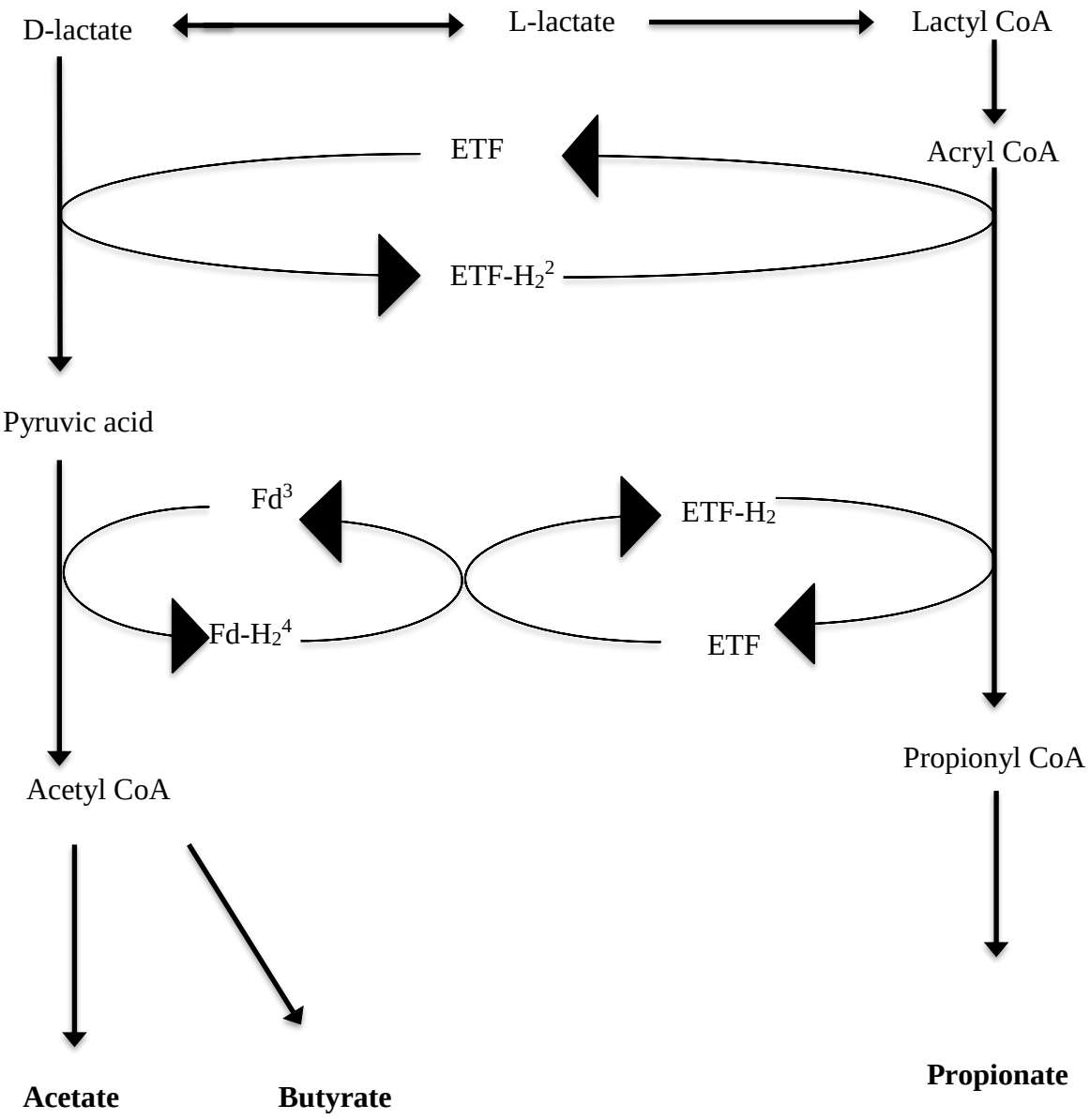


Figure 1.3 Schematic of lactate metabolism via the acrylate pathway



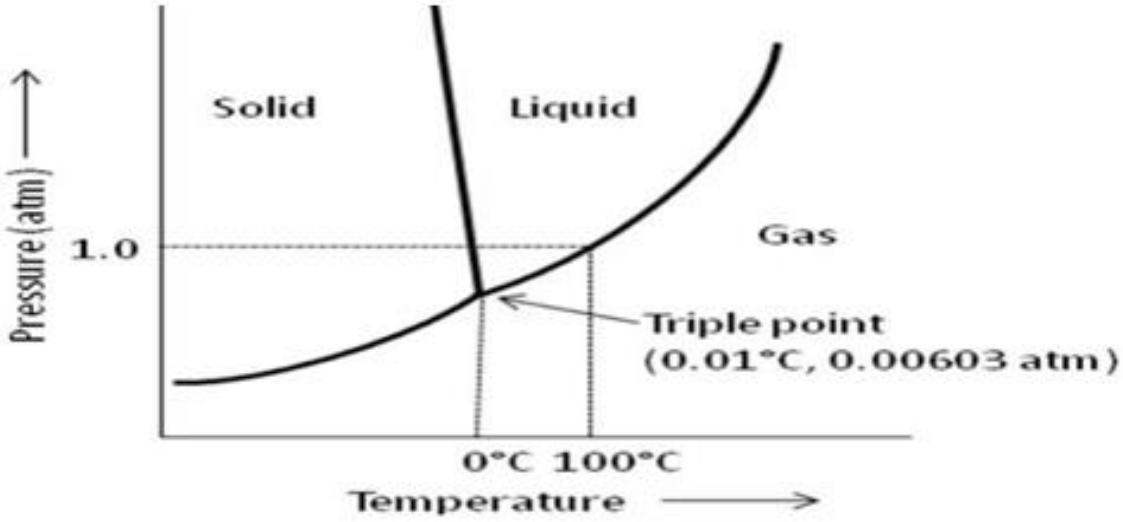
[1] ETF = electron-transferring flavoprotien

[2] ETF –H_2 = reduced form of electron-transferring flavoprotien

[3] Fd = ferredoxin

[4] Fd-H_2 = reduced form of ferredoxin

Figure 1.4 Phase diagram illustrating the triple point of water



[1]Lyophilization is carried out below the triple point, the chamber pressure must be below the vapor pressure of ice within sample for sublimation to occur.

[2]Adapted from http://www.pharmatutor.org/articles/lyophilization-process-overview

Chapter 2 - Ruminal characteristics and feedlot performance of steers during accelerated step-up to high-concentrate diets using *Megasphaera elsdenii* (Lactipro advance)

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Introduction

Adapting ruminal microbes to finishing diets is crucial to productivity of animals in feedlots. During this time, a shift from fiber digesting microbial populations to microorganisms capable of fermenting starch, soluble sugars, and lactic acid occurs (Nagaraja and Lechtenberg, 2007). Increasing proportions of rapidly fermentable carbohydrates in diets can lead to depressions in ruminal pH due to accumulation of lactic acid if produced in excess relative to metabolic capacity of lactic acid utilizing bacteria (LUB), epithelial absorption, and ruminal buffering. This condition, commonly known as acute ruminal acidosis, is characterized by ruminal pH less than 5.0 and lactic acid concentration above 50 mM (Nagaraja & Titgemeyer, 2007). Counotte et al. (1981) reported *Megasphaera elsdenii* metabolizes 60 to 80% of ruminal lactic acid, making it the dominant LUB naturally present within the rumen. Lactipro advance is a probiotic consisting of *M. elsdenii* NCIMB 41125. Currently, Lactipro *advance* has a short 14-d shelf life, and is administered as an oral drench at initial processing, and in many operations also at re-implanting. Preservation of the product via freeze-drying could potentially extend shelf life and allow for administration as an in-feed additive, thereby reducing labor associated with drenching by oral gavage, which would also extend the protection of *M. elsdenii* throughout the feeding period. Brown et al. (2014) reported *ad libitum* access to feed during adaptation periods of less than 14 d negatively impacts feedlot performance, but with the use of Lactipro advance, accelerated step-up regimens have been shown to be feasible (Drouillard et al., 2012; Miller, 2013). Objectives of this study were to: 1) determine impact of oral administration of *M. elsdenii* on ruminal characteristics and feedlot performance in steers subjected to accelerated step-up programs, 2) evaluate rehydrated freeze-dried oral drench as compared to Lactipro advance, and

3) determine if in feed administration in addition to an initial rehydrated, freeze-dried oral drench offers additional benefit

Materials and Methods

Animals and Sampling

This study was conducted at the Kansas State University Beef Cattle Research Center, located in Manhattan, Kansas. The Kansas State University Institutional Animal Care and Use Committee approved protocols and procedures for the study.

Experimental Design

This study was conducted as a randomized complete block design with 4 treatments. Crossbred steers (N = 435; initial BW 409 ± 5.5 kg) were blocked by weight and randomly assigned within block, to pens. Treatments consisted of a control which received no *M. elsdenii*, an initial fresh oral drench of Lactipro advance (Fresh), an initial oral gavage of rehydrated lyophilized culture (Rehyd), or an initial rehydrated oral gavage combined with lyophilized culture powder administered daily as a ration top-dress (Rehyd+Daily). Control cattle were stepped up with a conservative 22-d step-up period while all *M. elsdenii* treatments utilized an accelerated, 10-d program (Figure 1). Six or seven steers were housed in each of 64 partially covered, concrete surfaced pens (36.5 m^2) equipped with fence line feed bunks and automatic water fountains shared between adjacent pens. Steers were fed *ad libitum* once daily at approximately 13:00.

Animal Processing and Handling

Upon arrival at the Kansas State University Beef Cattle Research Center, steers were allowed *ad libitum* access to ground brome hay and water. Within 48 h of arrival, identification tags were placed in each ear, cattle were vaccinated with Ultrabac 7 (Zoetis, Parsippany, NJ) and

Bovi-shield Gold 5 (Zoetis), and individual BW were obtained. Steers were then offered *ad libitum* access to 50:50 brome to alfalfa hay diet until study initiation 48 hours after all cattle were received and processed. At the initiation of the study steers were implanted with Component TE-200 with Tylan (200 mg trembalone acetate and 20 mg estradiol; Elanco, Greenfield, IN) and oral gavage treatments were administered. Lactipro *advance* was drenched in 50 mL aliquots to the fresh treatment using a 50 mL Powermaster variable dose oral drench applicator (NJ Phillips, Grosford , Australia) delivering a minimum of 1.00×10^{10} CFU/animal, the lyophilized product was rehydrated in individual vials and administered via a 20-mL syringe to cattle in the Rehyd and Rehyd+Daily treatments (average of 1.00×10^{10} CFU/animal), and the control received no *M. elsdenii*. Lyophilized product was mixed with ground corn and top-dressed to the Rehyd +Daily treatment to provide an average of 2.19×10^{8} CFU/animal/d), while other treatments received a top-dress of ground corn. Steers were re-implanted on day 96 with Component TE-200 with Tylan (Elanco). Body weights were obtained for each pen at 28-day intervals and at the end of the finishing period. Average daily gains were computed by subtracting the initial BW from the final BW and dividing by d on feed.

Diet Preparation and Delivery

Composition of study diets is presented in Table 2.1. Steers were transitioned to their finishing diets in 10 d for the Fresh, Rehyd, and Rehyd+Daily treatments and in 22 days for the Control treatment. Step-up diets included 50:50 mixture of corn silage and alfalfa hay, providing progressively less forage with each step (40, 30, and 20% forage), and the finishing ration contained 10% corn silage and 90% concentrate. Feed was mixed once daily and hand delivered to pens. Lyophilized *M. elsdenii* was mixed in a ground corn carrier and top dressed at 0.5 lb per head, treatments not receiving the freeze-dried *M. elsdenii* were top dressed 0.5 lb per head of

only ground corn. Rations were mixed daily and delivered to bunks at approximately 1300 h. Feed intakes were monitored visually and adjusted daily to maintain minimal amounts of unconsumed feed the following day. Feed deliveries were recorded daily and unconsumed feed was collected every 28 days or as needed during the 28-day feeding intervals for determination of DMI. Subsamples of unconsumed feed was weighed, and dried in a 55°C oven for 48 h for determination of DM content. Dry matter intake was computed as DMI = [(total feed offered × % DM) – (total refused feed × % DM) / (number of animals × d).

Evaluation of Ruminal Fermentation Characteristics

Approximately 26 h after receiving the first diet, a 15-mL sample of ruminal fluid was extracted from 3 randomly selected steers from each of 32 pens (96 total animals, 24 per treatment) via rumenocentesis and immediately analyzed for pH with an Orion 2 Star Portable pH meter (Thermo Scientific, Waltham, MA). Four mL of ruminal fluid were mixed with 1 mL of 25% metaphosphoric acid and were frozen for later analyses of lactate and VFA concentrations. Ten Hungate tubes containing 10 mL of a semi-defined lactate medium were inoculated with 0.1 mL of ruminal fluid for each steer. Optical density of 2 tubes was measured on the Spectronic 20D+ (Thermo Electron Corporation, Beverly) at 6-h intervals over a 24-h incubation period and immediately frozen for later analysis of residual lactate concentration and 4 mL of each culture was combined with 1 mL of 25% metaphosphoric acid and frozen for analysis of VFA concentration. L-lactate concentration was measured on the YSI 2700 Select (YSI Inc., Yellow Springs, OH). Samples for VFA analysis were thawed and 2 mL of sample was transferred to micro centrifuge tubes for centrifugation at 17,000 x g for 10 min. Supernatant was removed and transferred to gas chromatography vials for storage at – 20°C and subsequent analysis. Analyses were performed on a 7890A gas chromatography system (Aligent

Technologies, Santa Clara, CA) with a standard obtained from Supelco (Supelco, Inc., Bellefonte, PA). A 15 m x 0.53 mm 25326 Nukol column was used for analyses with Hydrogen as carrier gas. Initial oven temperature was set at 100°C and incrementally increased 10°C per minute to a final temperature of with a 2 minute hold. Flow rate was 5.1 mL per minute and velocity was 45 cm per second.

Continuous pH data collection

Indwelling, wireless radiofrequency pH boli (length: 12 cm, width: 3.5 cm, weight: 210g; smaXtec pH Bolus SX-1042, smaXtec animal care, GmbH, Graz, Austria) were orally administered (using a balling gun provided by smaXtec animal care) to 1 steer from each of 32 pens (8 steers per treatment). These probes measured reticuloruminal pH every 10 min for 124 d with data collected by external repeaters (smaXtec animal care) evenly spaced throughout study pens and stored by a smaXtec US-3046 base station (smaXtec animal care). All data was accessed via smaXtec messenger 3.0 computer software (smaXtec animal care). Time spent under important pH thresholds was computed by multiplying number of readings under specific pH thresholds by 10 to define total minutes, then dividing by 60 to calculate total hours within different time periods (adaptation period d 1 to 28, adaptation through re-implanting d 29 to 96, and re-implanting through finishing d 97 to 124). Due to battery failure of several boli late in the finishing period, 124 d were used in the analysis of continuous ruminal pH. Nagaraja and Titgemeyer (2007) defined sub acute ruminal acidosis (SARA) as ruminal fluid with a pH between 5.0 and 5.6, and acute ruminal acidosis as less than pH 5.0. These values were used to establish important pH thresholds of between 5.6 and 5.2, 5.2 and 5.0, and under 5.0 for analyses in this study.

Slaughter

Final BW were determined on d 156, immediately before transporting steers 451 km to a commercial abattoir in Holcomb, KS. On the day of slaughter, animal identification, hot carcass weight, as well as incidence and severity of liver abscesses were recorded. Liver abscesses were scored according to the Elanco scoring system (Liver Abscess Technical Information AI 6288; Elanco Animal Health, Greenfield, IN: 0 = no abscess; A^- = 1 or 2 small abscess or abscess scars; A^0 to 4 small, well-organized abscesses; and A^+ = 1 or more large or active abscesses with or without adhesions). After 48 h of refrigeration, yield grade and USDA quality grade, marbling score, 12^{th}-rib fat thickness, and LM area were collected via camera image provided by the abattoir (VBG 2000: E+V Technology GmbH & Co. KG, Oranienburg, Germany).

Statistical analyses

Non-categorical data (live animal performance, HCW, yield grade, marbling score, 12^{th}-rib fat thickness, and LM area, as well as ruminal fluid pH, VFA, lactate concentrations, and continuous ruminal pH) were analyzed using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC). Categorical data (severity of liver abscesses, distribution of yield grades and USDA quality grades) were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Inst. Inc.). Experimental unit was pen, fixed effect was treatment, and random effect was block. *In vitro* optical density, L-lactate concentration, and VFA concentrations of semi defined lactate medium inoculated with mixed ruminal microbes also were analyzed with the MIXED procedure of SAS with experimental unit of animal, fixed effects were treatment, time, and treatment by time interaction, random effect of block, and repeated measure of time. Least

square means were separated using the PDIFF option. Differences among means were determined to be significant at an α level ≤ 0.05.

Results and Discussion

Steers were removed from the control (5), Rehyd (5), and Rehyd+Daily (2) due to injuries or death not related to treatment. Three steers were removed from the Fresh treatment group due to defective identification tags that were lost on the day of sorting.

Feedlot performance

Accelerated transition to finishing diets resulted in similar 28-day body weights ($P = 0.53$; Table 2.2), average daily gain ($P = 0.71$), and feed efficiency ($P = 0.69$) among treatments. Cattle in Rehyd+Daily treatment consumed less dry matter than those in the Control treatment ($P = 0.05$). Analogous intermittent and final feedlot performance was observed ($P > 0.20$) among all treatments. Studies have consistently shown no significant differences in final BW, ADG, or G:F between steers dosed with *M. elsdenii* compared to controls stepped-up with a variety of transition regimens and diet compositions (Leeuw et al., 2009; Henning et al., 2010; Drouillard et al., 2012; Miller, 2013). A review conducted by Brown et al. (2006) suggests that feeding cattle with *ad libitum* access to high-concentrate diets during transition to finishing diets will generally be followed by a decrease in feed intake, and that step-up regimens of less than 14 d adversely affect feedlot performance. Cattle treated with *M. elsdenii* have been reported to have higher DMI in the days after accelerated step-up regimens than cattle not receiving *M. elsdenii* (Henning et al., 2010; Miller, 2013), while other studies have reported no differences (Meissner et al., 2010; Miller, 2013). With a 22-d step-up period for control cattle, similarities among treatments in this study indicate accelerated step-up programs can be implemented using any of the three forms of *M. elsdenii* treatments without adversely affecting feedlot performance.

Carcass characteristics

The effects of *M. elsdenii* on carcass characteristics are presented in Table 2.3. There were no differences among treatments in HCW ($P = 0.63$), dressing percentage ($P = 0.31$), 12th rib subcutaneous fat thickness ($P = 0.12$), or LM area ($P = 0.66$). Regardless of roughage level or step-up regimen, literature supports similarities found in this study (Leeuw et al., 2009; Miller, 2013). Although not significant, *M. elsdenii* also has been reported to increase HCW by 2.3 to 9.4% (Drouillard et al., 2012). Fresh and Rehyd+Daily treatments resulted in fewer carcasses grading yield grade 1 ($P = 0.03$, $P = 0.008$) and yield grade 5 ($P = 0.02$, $P = 0.01$) than the Rehyd (Table 2.3). No dissimilarities were observed amid treatments in yield grades 2, 3, or 4 ($P > 0.40$). This observation is consistent with findings of Miller (2013), in which cattle dosed with fresh cultures of *M. elsdenii* had reduced incidence of YG 1, which they attributed to increased time on the finishing diet compared to control cattle. USDA quality grades were similar among treatments ($P > 0.05$, Table 2.3). Liver abscess occurrence and severity were similar among treatments even with no Tylan (Elanco Animal Health) included in the diet ($P = 0.56$, $P > 0.30$, respectively; Table 2.3).

Continuous ruminal pH

No treatment x period interactions were detected in this study ($P > 0.05$; Figure 2.2-2.4), but main effects of treatment ($P < 0.01$) and period ($P < 0.01$) were found. During adaptation (d 1 to 28; Figure 2.2), there were no treatment differences among the 3-pH thresholds ($P > 0.05$). Between adaptation and re-implanting (d 29 to 96; Figure 2.3), Rehyd treatment spent 43% less time with ruminal pH between 5.2 and 5.6 than the Fresh treatment ($P = 0.04$). Rehyd+Daily reduced time spent in the same pH threshold by 86 % compared to Fresh ($P < 0.01$), 80% when compared to Control ($P < 0.01$), and 17% compared to Rehyd ($P < 0.04$).

Steers receiving Rehyd+Daily treatment spent an average of 1 h within the ruminal pH range of 5.0 to 5.2 in the 68 d period between acclimation to finishing diets and re-implanting. This translates to a 98% reduction as compared to both Control and Fresh treatments ($P = 0.03$). No differences were found in this time period for pH below 5.0 ($P > 0.10$). Figure 2.4 illustrates differences in time spent within important pH thresholds for the last 28 d pH probes measured ruminal pH. Control and Fresh cattle spent 85 and 90% respectively more time late in the finishing period with a ruminal pH between 5.2 and 5.6 as compared to Rehyd+Daily ($P < 0.05$). Cattle in the Rehyd and Rehyd+Daily treatments spent 85 and 99% less time between pH 5.0 and 5.2 than Controls ($P < 0.01$), and Rehyd+Daily spent 98% less time in the same pH threshold than Fresh cattle ($P = 0.02$). Rehyd treated cattle spent an average of 1 h in a state of acute acidosis and Rehyd+Daily did not encounter a full hour over the last 28 d of pH monitoring. Control and Fresh cattle spent significantly more time in acute acidosis during this time period (98 h, $P < 0.01$ and 67 h, $P = 0.01$ respectively).

Effects of time period were detected for the pH thresholds of 5.0 to 5.2 and pH under 5.0 ($P < 0.01$), but no effect was observed for pH between 5.2 and 5.6 ($P > 0.10$). Similar incidence and severity of pH between 5.0 and 5.2 as well as pH under 5.0 were observed for the acclimation period (d 1 to 28; $P > 0.01$) and between acclimation and re-implanting (d 29 to 96; $P < 0.01$). The last 28 d of pH measurement reveals an increase in prevalence of these 2 pH thresholds relative to those of the first 96 d of the study. For the percent of time spent between ruminal pH of 5.0 and 5.2, the final 28 d increased by 84% relative to the first 28 d ($P < 0.01$), and 64% when compared to d 29 to 96 ($P < 0.01$). Proportion of time spent under pH 5.0 in inflated by 97.6% in comparison to the transition period ($P < 0.01$), and 91.1% in comparison to the 68 d between acclimation to feedlot diets and re-implanting ($P < 0.01$).

McDaniel (2009) found steers dosed with fresh cultures of *M. elsdenii* maintained higher ruminal pH than control steers throughout the 24 h period after introduction of fermentable carbohydrates to the rumen, but no response was observed 48 h after inoculation. This suggests fresh cultures of *M. elsdenii* are able to protect the rumen against acidosis if drenched relatively close to the time of diet changes. In the present study, drenching steers with *M. elsdenii*, fresh or rehydrated, proved effective in minimizing the time cattle spent in an acidotic state while transitioning to finishing diets 14 d faster than controls. Increased DMI has been identified as a risk factor for acidosis (McDaniel, 2009; Castillo-Lopez et al., 2014). Castillo-Lopez et al. (2014) reported a positive correlation between DMI and time spent under pH 5.5, indicating cattle that consume more DM are more likely to spend time under pH 5.5. In the case of this study, steers in the Control group consumed more DM than Rehyd+Daily steers but no differences in ruminal pH were observed. This increase in DMI is more likely due to differences in step-up regimens (Figure 2.1) than time spent under ruminal pH thresholds. Nagaraja and Lechtenberg (2007) and Castillo-Lopez et al. (2014) reported cattle are not only at risk of acidosis during the adaptation period but are also at risk in the late finishing period. Increasing DMI as cattle grow introduces an increased amount of fermentable carbohydrates to the rumen micro flora, possibly causing accumulation of either VFAs or lactic acid (Castillo-Lopez et al., 2014). In the present study, an increase in the proportion of time steers spent between pH 5.0 and 5.2 and under pH 5.0 for the Control and Fresh treatments in the late finishing period could be attributed to increased DMI. The added protection of daily administration of *M. elsdenii* may be an explanation as to why Rehyd+Daily steers are not impacted as much by time period.

Characteristics of ruminal fluid

Results of pH, VFA profile, and lactate concentration of the ruminal fluid extracted are presented in Table 2.4. No differences in ruminal pH or lactates were detected ($P = 0.33$). There was a difference in Total VFA concentration of ruminal fluid with the Rehyd+Daily treatment being significantly higher than the other treatments ($P < 0.01$). Rehyd+Daily treatment had higher acetate concentration than the Rehyd treatment ($P = 0.02$). Although there was a significant difference in acetate concentration, the acetate:proprionate ratio was similar among treatments ($P = 0.96$). The means for all other VFAs and lactic acid were similar among treatments ($P > 0.18$). In this study ruminal pH was above 6.0 for all treatments at the time of sampling, which corresponds with the low concentrations of lactic acid of the ruminal fluid. Although, total VFA is slightly lower than the normal concentration reported by Nagaraja and Titgemeyer (2006) of 100 mM. McDaniel (2009) utilized cannulated steers and found greater depression in pH and increased lactate concentration in control cattle within the first 24 h after steers received an initial grain challenge ($P < 0.05$). Increased concentrations of isovalerate, valerate, and total VFA were also detected when cattle were dosed with *M. elsdenii* relative to controls ($P < 0.05$; McDaniel, 2009). Increasing CFU provided to the animal has previously lead to no differences in pH, VFAs, or lactate concentration ($P > 0.05$, Henning et al., 2010; McDaniel, 2009). Henning et al. (2010) also reported a trend for *M. elsdenii* dosed cattle to have increased proportions of acetic and butyric acids and lower propionic acid. Location within the rumen and timing of sampling after feeding can result in very different conclusions, therefore inconsistencies with previous research may be due to one of these factors.

In vitro characteristics

Optical density measurements were observed in 6-h intervals over a 24-h period to determine the growth curves of mixed ruminal microbes inoculated into a semi-defined lactate medium, these data are presented in Figure 2.5. There was a treatment × time interaction detected ($P < 0.02$). Effects of treatment ($P < 0.01$) and time ($P < 0.01$) were also found. No differences among treatments were detected until h 12, where increased growth of Rehyd was observed in comparison to Rehyd+Daily ($P < 0.02$) and Control ($P < 0.01$). At h 24, there were no differences between *M. elsdenii* treatments ($P > 0.10$), but control treatment had significantly less microbial growth than all *M. elsdenii* treatments ($P < 0.01$).

Figure 2.6 illustrates L-lactate disappearance from semi-defined lactate medium inoculated with mixed ruminal microbes and incubated for 0, 6, 12, 18, or 24 h. A treatment × time interaction was detected ($P < 0.01$) along with effects of treatment ($P = 0.01$) and time of incubation ($P < 0.01$). Fresh treatment contained less L-lactate than other treatments at h 0 ($P = 0.04$). Concentrations of L-lactate were analogous among treatments over the 6, 12, and 18-h time points ($P > 0.10$). Similar to the results of the optical density, the ruminal microbes from control steers utilized less lactate ($P < 0.01$) than the *M. elsdenii* treatments collectively at 24 h of incubation, while no differences were detected among *M. elsdenii* treatments ($P > 0.10$). Weimer & Moen (2013) reported that *M. elsdenii* T81 depleted nearly all of the lactate when inoculated into semi-defined lactate media with concentrations of 50 to 210 mM lactic acid. Ruminal microorganisms of steers drenched with *M. elsdenii* utilized lactic acid more efficiently *in vitro* than placebos for up to 72 h post inoculation, a direct relationship between *M. elsdenii* concentration and capacity of ruminal microbes to utilize lactic acid was also detected by McDaniel (2009). These data, paired with previous research, illustrate that ruminal microbes

from steers treated with *M. elsdenii* grew more efficiently in a semi-defined lactate medium than those from control steers, suggesting greater capacity for lactic acid utilization in these treatments. Moreover, *M. elsdenii* treatments were similar with respect to capacity for utilization of L-lactic acid. The ability of this bacterium to utilize lactic acid to produce VFAs makes it a viable option for mitigation of ruminal acidosis.

VFA concentrations were measured on the samples used for growth and lactate disappearance assays and are presented in Table 2.5. There was a treatment × time interaction detected for the concentrations of total VFA ($P < 0.01$), acetate ($P < 0.01$), isobutyrate ($P = 0.04$), butyrate ($P < 0.01$), isovalerate ($P < 0.01$), and valerate ($P < 0.01$), as well as for the acetate:propionate ratio ($P = 0.02$). Effects of time were found for total VFA and all individual VFAs ($P < 0.01$). Differences among treatments were found for isobutyrate ($P = 0.02$), butyrate ($P < 0.01$), and valerate ($P < 0.01$). Concentrations of isobutyrate were lower at h 18 for Fresh and Rehyd+Daily treatment groups ($P < 0.01$) when compared to the control and Rehyd. At 24-h of incubation Rehyd+Daily had less isobutyrate ($P < 0.01$) than Fresh and Rehyd treatments but was similar to control ($P > 0.10$). Butyrate concentrations of Rehyd samples were higher than that of Rehyd+Daily ($P = 0.02$) after 18 h of incubation. Control treatment produced less butyrate in 24 h than *M. elsdenii* treatments ($P < 0.01$). Differences in butyrate were also detected among *M. elsdenii* treatments at this time point with Fresh samples containing higher concentration than Rehyd treatment ($P = 0.01$). Hour 18 revealed reduced valerate concentration for Rehyd when compared to other treatments ($P = 0.01$). Similar observations were made for valerate and butyrate concentration at 24 h of incubation. Controls contained less valerate than the average of *M. elsdenii* treatments ($P < 0.01$), while concentrations were higher in Fresh samples than in Rehyd ($P = 0.03$). No main effect of treatment was detected for other VFAs ($P >$

0.05), and negligible concentrations of isocaproate, caproate, and heptanoate were measured. A treatment × time interaction ($P = 0.02$) was found for the acetate:propionate ratio as well as an effect of time ($P > 0.01$), but was similar among treatments ($P > 0.10$).

Megasphaera elsdenii has been reported to produce quantitatively similar amounts of acetate and propionate, and butyrate and valerate respectively with trace amounts of caproate, *in vitro* (Weimer and Moen, 2013). Increased production of isovalerate, as well as lower A:P over controls were found by McDaniel (2009) when inoculating semi-defined lactate medium with ruminal microorganisms 3 d post drenching, as well as increased valerate production for up to 42 d post ruminal inoculation. Kung and Hession (1995) found increased concentrations of total VFA, propionate, isobutyrate, butyrate, isovalerate, and valerate when simulating ruminal acidosis *in vitro*. In the current study, increases in butyrate and valerate were observed for M. elsdnii treatments. Ninety percent of butyrate is metabolized by ruminal epithelium (Bergman, 1990), and what bypasses the rumen serves as a preferred energy source for intestinal epithelium. Valerate is a 5 carbon VFA produced by one of two pathways: 1) Stickland reactions from proline or 2) from acetyl coA and propionyl coA. Elsden and Lewis (1953) were intrigued by *M.elsdenii* because at the time it was discovered relatively few microorganisms produced VFAs with carbon chains exceeding that of butyrate (4 carbons). Although no differences were found between treatments, acetate and propionate are the predominant VFAs produced by *M. elsdenii*. Acetate may serve as a precursor for lipid synthesis (Hanson and Ballard, 1967), or can be absorbed by the ruminal epithelium, metabolized in the liver and serve as a major energy source for body tissues (Ballard, 1972). A unique property of propionate is its gluconeogenic nature, making this VFA very important for gluconeogenesis in the liver. While *M. elsdenii* is known for its major role in metabolism of lactate and other carbohydrates, such as glucose, and fructose, it

also is proteolytic. The BCAAs valine, leucine, and isoleucine are metabolized to BCVFAs isobutyrate, isovalerate, and 2-methylbutyrate, respectively (Allison et al., 1979). Branched chain volatile fatty acids improve fiber digestion by providing growth factors for fibrolytic bacteria in the rumen.

Conclusions

All three forms of *M. elsdenii* can make achieving an accelerated step-up program possible without negatively affecting feedlot performance or carcass traits. Due to minimal lactate concentrations at the time of rumenocentesis, no differences in ruminal pH or VFA concentrations were detected. Ruminal fluid containing fresh cultures or freeze-dried and rehydrated cultures of *M. elsdenii* NCIMB 41125 were equally effective in metabolizing lactic acid. In this experiment, daily administration of lyophilized *M. elsdenii* resulted in less time spent in an acidotic state, but no further benefits were realized.

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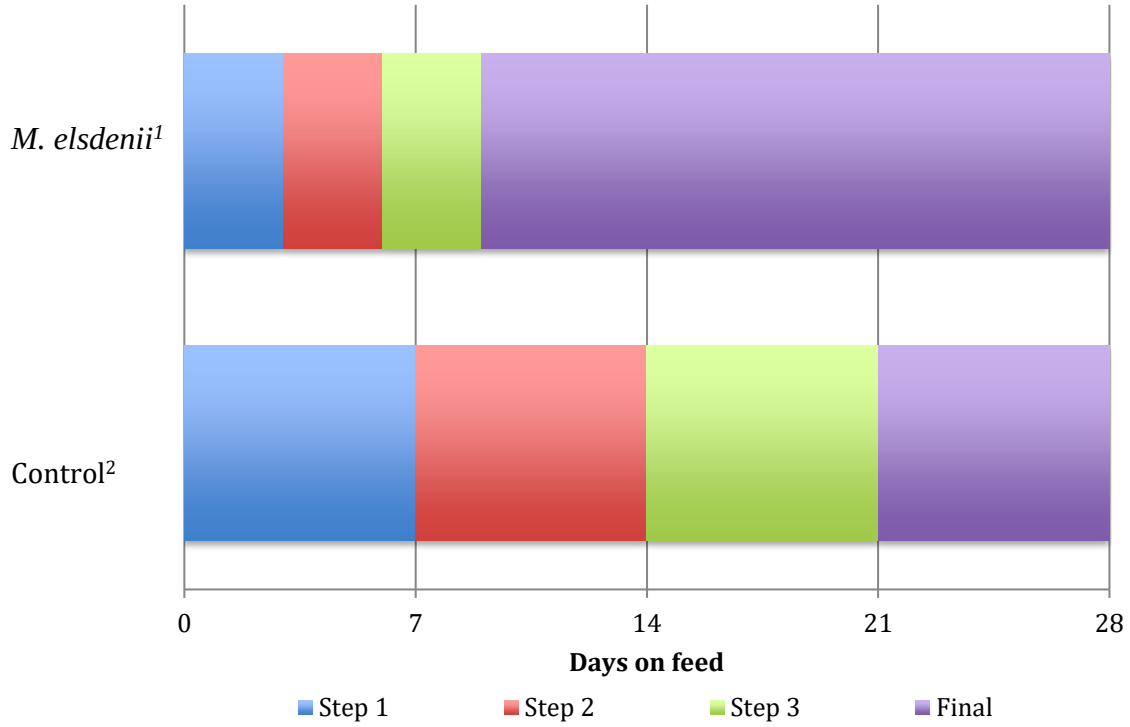
Figure 2.1 Step-up regimens



[1] Step-up regimen for Fresh, Rehyd, and Rehyd+Daily treatments utilized 3 transition diets fed for 3 d each before the final diet was fed on d 10

[2] Step-up regimen for Control treatment utilized 3 transition diets fed for 7 d each before the final diet was fed on d 22

Table 2.1 Diet composition

Item, % DM basis	Step 1	Step 2	Step 3	Final
Corn Silage	20.00	15.00	10.00	10.00
Wet corn gluten feed	30.00	30.00	30.00	30.00
Steam flaked corn	25.63	35.63	45.63	55.63
Alfalfa hay	20.00	15.00	10.00	0.00
Ground corn[1]	2.20	2.20	2.20	2.20
Vitamin/trace mineral premix[2]	2.17	2.17	2.17	2.17
Analyzed nutrient composition				
Neutral detergent fiber, %	29.39	26.07	22.76	18.66
Crude protein, %	14.91	14.72	14.54	14.00
Calcium, %	0.97	0.89	0.82	0.70
Phosphorus, %	0.47	0.47	0.48	0.49

[1] Freeze-dried *M. elsdenii* mixed with ground corn carrier for Rehyd+Daily treatment, other treatments received ground corn

[2] Formulated to provide the following in the total diet DM: : 0.15 mg/kg Co, 10 mg/kg Cu, 0.5 mg/kg I, 20 mg/kg Mn, 0.10 mg/kg Se, 30 mg/kg Zn, 33 mg/kg monensin (Rumensin, Elanco Animal Health), 2200 IU/kg added vitamin A, and 51 IU/kg added alpha tocopherol acetate

Table 2.2 Feedlot performance of steers dosed with fresh or freeze-dried, rehydrated *Megasphaera elsdenii* and placed on accelerated step-up regimens

Item	Control	Fresh	Rehyd	Rehyd+ Daily	SEM	*P*-value
Initial BW, lb	409	408	408	408	5.19	0.74
Day 1 to 28						
BW d 28, lb	480	478	476	477	5.94	0.53
DMI, lb	9.95[a]	9.59[a,b]	9.66[a,b]	9.37[b]	0.18	0.05
ADG, lb	2.54	2.49	2.31	2.44	0.08	0.71
G:F	0.2559	0.2596	0.2489	0.2594	0.007	0.69
Day 29 to 96						
BW d 96, lb	591	594	592	588	4.65	0.58
DMI, lb	11.19	11.23	11.31	11.20	0.15	0.83
ADG, lb	1.63	1.71	1.71	1.62	0.06	0.43
G:F	0.1459	0.1526	0.1510	0.1466	0.005	0.47
Day 97 to 156						
DMI, lb	11.47	11.89	12.08	11.70	0.24	0.08
ADG, lb	1.87	1.95	1.95	1.97	0.08	0.57
G:F	0.1691	0.1647	0.1617	0.1705	0.004	0.15
Day 1 to 156						
BW d 156, lb	707	712	709	707	7.12	0.84
DMI, lb	11.00	11.23	11.28	11.05	0.15	0.29
ADG, lb	1.91	1.95	1.93	1.92	0.03	0.78
G:F	0.1743	0.1733	0.1705	0.1732	0.003	0.66

[†] Control received no *M. elsdenii* and utilized a 21-d step-up; Fresh received an upfront fresh oral drench of Lactipro *advance* (MS Biotec, Wamego, KS) with a 9-d step-up; Rehyd, received an upfront freeze-dried, rehydrated oral drench with a 9-d step-up; Rehyd+Daily upfront freeze-dried, rehydrated oral drench with daily in feed administration and utilized a 9-d step-up

[a,b] Means within a row without a common superscript letter are different, $P < 0.05$

Table 2.3 Carcass characteristics of carcasses from steers dosed with fresh or freeze-dried, rehydrated *Megasphaera elsdenii* and placed on accelerated step-up regimens

Item	Control	Fresh	Rehyd	Rehyd+ Daily	SEM	*P*-value
HCW, lb	434	436	439	435	4.29	0.63
Dressed yield, %	61.3	61.2	61.7	61.5	0.21	0.31
Total liver abscess, %	23.03	18.75	26.37	20.71	4.28	0.56
Mild, %	15.88	13.24	19.16	11.38	3.66	0.38
Moderate, %	3.54	3.74	2.73	4.33	1.86	0.94
Severe, %	3.63	1.78	4.48	4.34	1.94	0.69
USDA yield grade						
1, %	3.54[a,b]	0.94[b]	5.50[a]	0.00[b]	1.50	0.04
2, %	23.94	27.46	24.97	25.20	4.38	0.94
3, %	50.38	55.52	44.81	51.28	4.96	0.47
4, %	18.59	16.05	20.19	23.48	3.88	0.57
5, %	3.54[a,b]	0.00[b]	4.59[a]	0.00[b]	1.36	0.02
Marbling score[1]	523	520	510	520	10.45	0.77
USDA quality grade, %						
Prime	8.08[a]	6.66[a]	1.90[b]	9.58[a]	2.59	0.01
Choice	84.91	88.74	93.61	82.62	3.37	0.07
High	15.04	17.92	11.93	10.43	3.35	0.38
Mid	26.52	27.40	37.55	27.89	4.57	0.24
Low	43.36	46.22	39.45	46.17	4.83	0.72
Select	5.29	3.64	3.58	6.92	2.27	0.61
Sub Select[2]	1.76	0.94	0.91	1.01	1.05	0.92
Premium, %[3]	49.09	50.97	52.66	46.58	5.00	0.82
12th rib subcutaneous fat thickness, cm	1.78	1.65	1.80	1.80	0.05	0.12
LM area, cm^2	101.42	100.84	100.52	99.48	1.23	0.68
Carcass value, $	1,679.67	1,704.22	1,698.68	1,672.28	17.8	0.46

† Control received no *M. elsdenii* and utilized a 21-d step-up; Fresh received an upfront fresh oral drench of Lactipro *advance* (MS Biotec, Wamego, KS) with a 9-d step-up; Rehyd, received an upfront freeze-dried, rehydrated oral drench with a 9-d step-up; Rehyd+Daily upfront freeze-dried, rehydrated oral drench with daily in feed administration and utilized a 9-d step-up

[a,b] Means within a row without a common superscript letter are different, $P < 0.05$

[1] Marbling score determined by camera imaging: Modest = 500 to 599

[2] Carcasses grading below USDA-Select

[3] Carcasses grading mid-Choice or higher

Table 2.4 Ruminal fluid characteristics 26 h after feeding initial diets

Item	Control	Fresh	Rehyd	Rehyd+Daily	SEM	*P*-value
Ruminal pH	6.21	6.03	6.13	6.00	0.13	0.33
Volatile fatty acid, mM						
Total	86.3[a]	88.1[a]	81.1[a]	93.5[b]	3.97	< 0.01
Acetate	54.1[a,b]	54.9[a,b]	50.8[a]	58.4[b]	2.40	0.02
Propionate	20.4	21.2	19.6	22.6	1.47	0.18
Butyrate	10.3	10.6	9.4	10.8	0.73	0.44
Isobutyrate	0.24	0.15	0.09	0.21	0.08	0.20
Valerate	0.58	0.67	0.64	0.64	0.10	0.93
Isovalerate	0.67	0.61	0.50	0.66	0.11	0.23
Caproate	0.00	0.05	0.00	0.02	0.02	0.28
A:P	2.74	2.67	2.68	2.72	0.11	0.96
Lactate, mM	0.78	0.75	0.72	0.75	0.09	0.54

[†] Control received no *M. elsdenii* and utilized a 21-d step-up; Fresh received an upfront fresh oral drench of Lactipro *advance* (MS Biotec, Wamego, KS) with a 9-d step-up; Rehyd received an upfront freeze-dried, rehydrated oral drench with a 9-d step-up; Rehyd+Daily upfront freeze-dried, rehydrated oral drench with daily in feed administration and utilized a 9-d step-up

[a,b] Means within a row without a common superscript letter are different, $P < 0.01$

Table 2.5 Changes in VFA profile of a semi-defined lactate medium inoculated with mixed ruminal microbes

	Treatments[†]					*P* – values		
Item	Control	Fresh	Rehyd	Rehyd+Daily	SEM	Trt	h	Trt x h
Total VFA, mM						0.36	< 0.01	< 0.01
0 h[1]	3.61	3.35	3.52	3.55	5.16			
6 h[1]	6.41	7.17	7.79	6.62	5.16			
12 h[2]	7.64	8.58	17.27	7.80	5.16			
18 h[3]	63.01	49.48	56.45	53.73	5.16			
24 h[4]	46.23	78.35	67.22	74.77	5.16			
Acetate, mM						0.98	< 0.01	< 0.01
0 h[1]	3.07	3.02	3.07	2.92	2.15			
6 h[1,2]	4.92	5.56	5.78	5.13	2.15			
12 h[2]	5.77	6.31	9.17	6.27	2.15			
18 h[3]	33.47	21.96	22.75	23.97	2.15			
24 h[4]	17.25	25.43	22.82	26.05	2.15			
Propionate, mM						0.24	<0.001	0.08
0 h[1]	0.01	0.00	0.00	0.00	2.44			
6 h[1,2]	1.21	1.29	1.69	1.21	2.44			
12 h[2]	1.69	2.00	6.45	1.47	2.44			
18 h[3]	23.29	20.84	23.53	24.00	2.44			
24 h[4]	20.85	33.61	28.77	32.58	2.44			
A:P						0.57	< 0.01	0.02
0 h[1]	0.06	0.00	0.00	0.00	0.18			
6 h[1,2]	4.01	4.44	4.24	4.00	0.18			
12 h[2]	3.98	4.60	4.17	4.60	0.18			
18 h[3]	2.14	1.91	1.77	1.91	0.18			
24 h[4]	1.12	0.82	0.91	0.91	0.18			

	Treatment[†]					*P*-value[‡]		
Item	Control	Fresh	Rehyd	Rehyd+Daily	SEM	Trt	h	Trt x h
Isobutyrate, mM						0.02	< 0.01	0.04
0 h[1]	0.03	0.00	0.00	0.00	0.03			
6 h[1]	0.01	0.00	0.00	0.00	0.03			
12 h[1]	0.00	0.00	0.02	0.00	0.03			
18 h[2]	0.14[a]	0.01[b]	0.15[a]	0.01[b]	0.03			
24 h[3]	0.15[a,b]	0.22[a]	0.22[a]	0.08[b]	0.03			
Butyrate, mM						< 0.01	< 0.01	< 0.01
0 h[1]	0.08	0.06	0.08	0.08	0.52			
6 h[1]	0.15	0.20	0.19	0.16	0.52			
12 h[1]	0.18	0.25	0.90	0.16	0.52			
18 h[2]	2.71[a,b]	3.24[a,b]	4.36[a]	2.75[b]	0.52			
24 h[3]	3.46[a]	8.80[b]	7.06[c]	7.46[b,c]	0.52			
Valerate, mM						< 0.01	< 0.01	< 0.01
0 h[1]	0.14	0.10	0.16	0.17	0.54			
6 h[1]	0.05	0.07	0.08	0.06	0.54			
12 h[1]	0.00	0.03	0.63	0.00	0.54			
18 h[2]	2.63[a]	2.91[a]	4.63[b]	2.53[a]	0.54			
24 h[3]	3.75[a]	8.12[b]	7.23[c]	7.56[b,c]	0.54			

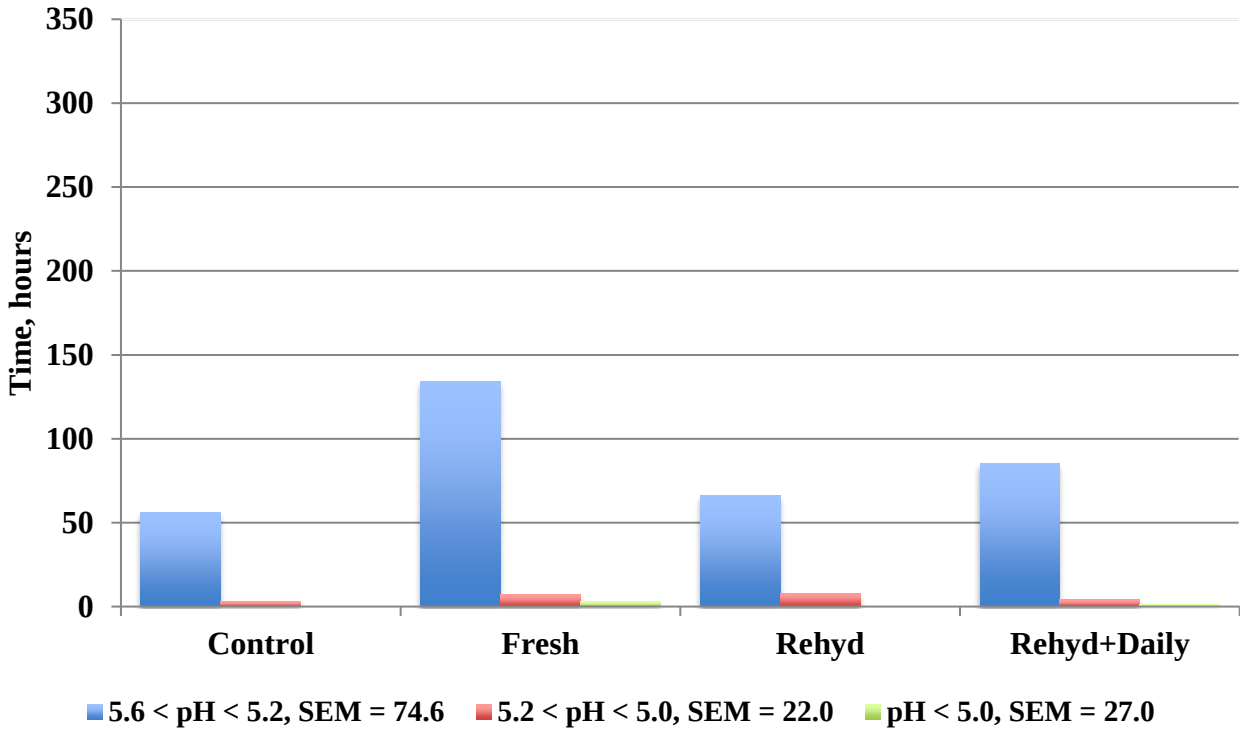
[†] Control received no *M. elsdenii* and utilized a 21-d step-up; Fresh received an upfront fresh oral drench of Lactipro advance (MS Biotec, Wamego, KS) with a 9-d step-up; Rehyd, received an upfront freeze-dried, rehydrated oral drench with a 9-d step-up; Rehyd+Daily upfront freeze-dried, rehydrated oral drench with daily in feed administration and utilized a 9-d step-up

[‡] Trt = treatment, h = hour of incubation, Trt × h = treatment by hour interaction

[1,2,3,4] Time points without a common superscript number are different, $P < 0.01$

[a,b,c] Means within a row without a common superscript letter are different, $P < 0.01$

Figure 2.2 Time spent under important pH thresholds during adaptation d 1 to 28



† Control received no *M. elsdenii* and utilized a 21-d step-up; Fresh received an upfront fresh oral drench of Lactipro *advance* (MS Biotec, Wamego, KS) with a 9-d step-up; Rehyd, received an upfront freeze-dried, rehydrated oral drench with a 9-d step-up; Rehyd+Daily upfront freeze-dried, rehydrated oral drench with daily in feed administration and utilized a 9-d step-up

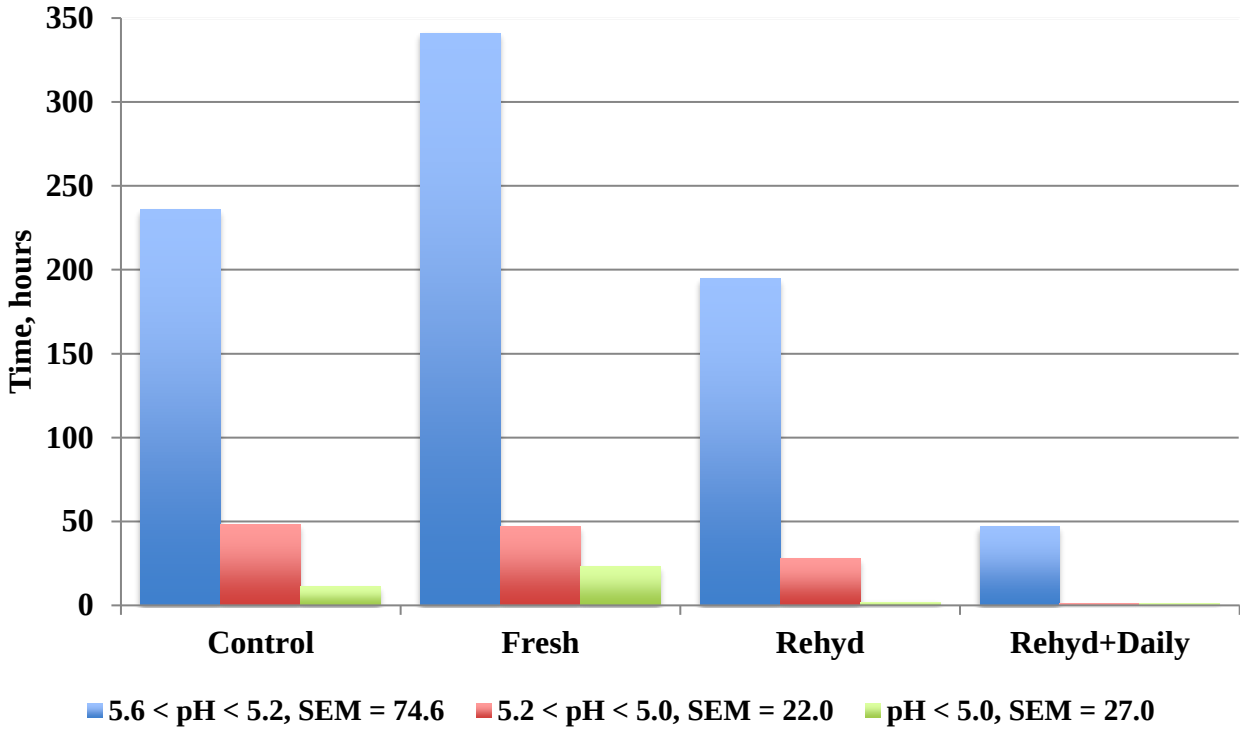
[1]Treatment x period, 5.6 > pH > 5.2, P > 0.10; 5.2 > pH > 5.0, *P* > 0.10; pH < 5.0, *P* = 0.06

[2] Treatment effect, 5.6 > pH > 5.2, P > 0.01; 5.2 > pH > 5.0, *P* < 0.01; pH < 5.0, *P* = 0.02

[3] Period effect, 5.6 > pH > 5.2, P > 0.01; 5.2 > pH > 5.0, *P* = 0.01; pH < 5.0, *P* < 0.01

[4] Single dose vs. control, 5.6 > pH > 5.2, P > 0.10; 5.2 > pH > 5.0, *P* = 0.08; pH < 5.0, *P* > 0.10 control versus average of Fresh culture and rehydrated culture administered as a single oral drench at initial processing

Figure 2.3 Time spent under important pH thresholds between adaptation and re-implanting d 29 to 96



† Control received no *M. elsdenii* and utilized a 21-d step-up; Fresh received an upfront fresh oral drench of Lactipro *advance* (MS Biotec, Wamego, KS) with a 9-d step-up; Rehyd, received an upfront freeze-dried, rehydrated oral drench with a 9-d step-up; Rehyd+Daily upfront freeze-dried, rehydrated oral drench with daily in feed administration and utilized a 9-d step-up

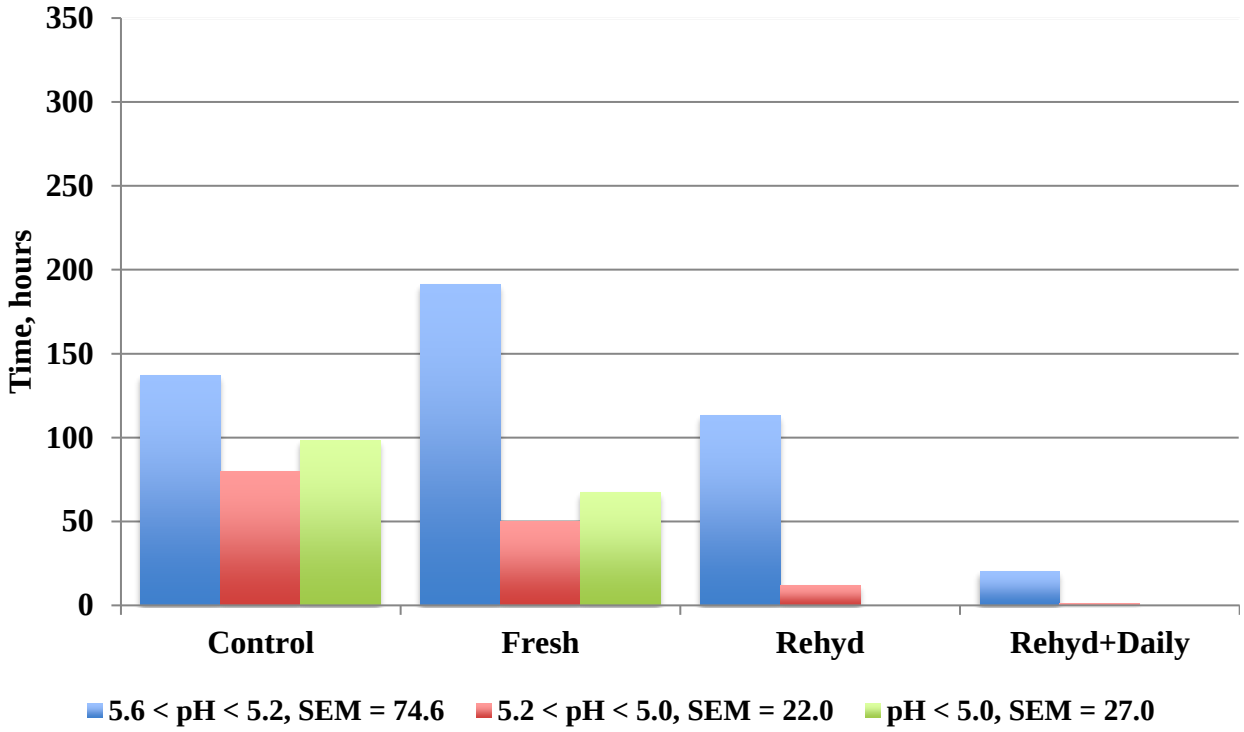
[1]Treatment x period, 5.6 > pH > 5.2, P > 0.10; 5.2 > pH > 5.0, *P* > 0.10; pH < 5.0, *P* = 0.06

[2] Treatment effect, 5.6 > pH > 5.2, P > 0.01; 5.2 > pH > 5.0, *P* < 0.01; pH < 5.0, *P* = 0.02

[3] Period effect, 5.6 > pH > 5.2, P > 0.01; 5.2 > pH > 5.0, *P* = 0.01; pH < 5.0, *P* < 0.01

[4] Single dose vs. control, 5.6 > pH > 5.2, P > 0.10; 5.2 > pH > 5.0, *P* = 0.08; pH < 5.0, *P* > 0.10 control versus average of Fresh culture and rehydrated culture administered as a single oral drench at initial processing

Figure 2.4 Time spent under important pH thresholds between re-implanting and finishing d 97 to 124



† Control received no *M. elsdenii* and utilized a 21-d step-up; Fresh received an upfront fresh oral drench of Lactipro *advance* (MS Biotec, Wamego, KS) with a 9-d step-up; Rehyd, received an upfront freeze-dried, rehydrated oral drench with a 9-d step-up; Rehyd+Daily upfront freeze-dried, rehydrated oral drench with daily in feed administration and utilized a 9-d step-up

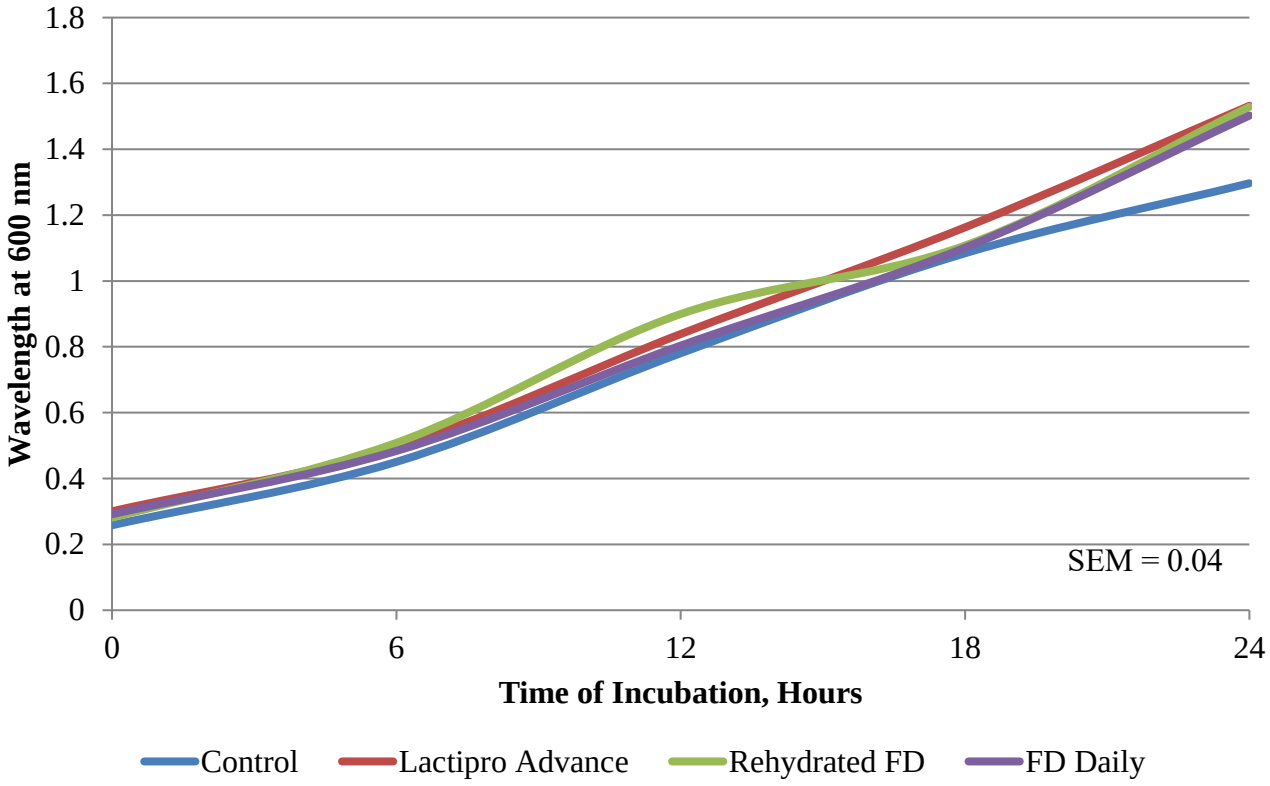
[1]Treatment x period, 5.6 > pH > 5.2, P > 0.10; 5.2 > pH > 5.0, *P* > 0.10; pH < 5.0, *P* = 0.06

[2] Treatment effect, 5.6 > pH > 5.2, P > 0.01; 5.2 > pH > 5.0, *P* < 0.01; pH < 5.0, *P* = 0.02

[3] Period effect, 5.6 > pH > 5.2, P > 0.01; 5.2 > pH > 5.0, *P* = 0.01; pH < 5.0, *P* < 0.01

[4] Single dose vs. control, 5.6 > pH > 5.2, P > 0.10; 5.2 > pH > 5.0, *P* = 0.08; pH < 5.0, *P* > 0.10 control versus average of Fresh culture and rehydrated culture administered as a single oral drench at initial processing

Figure 2.5 Changes in optical density of a semi-defined lactate medium inoculated with mixed ruminal microbes



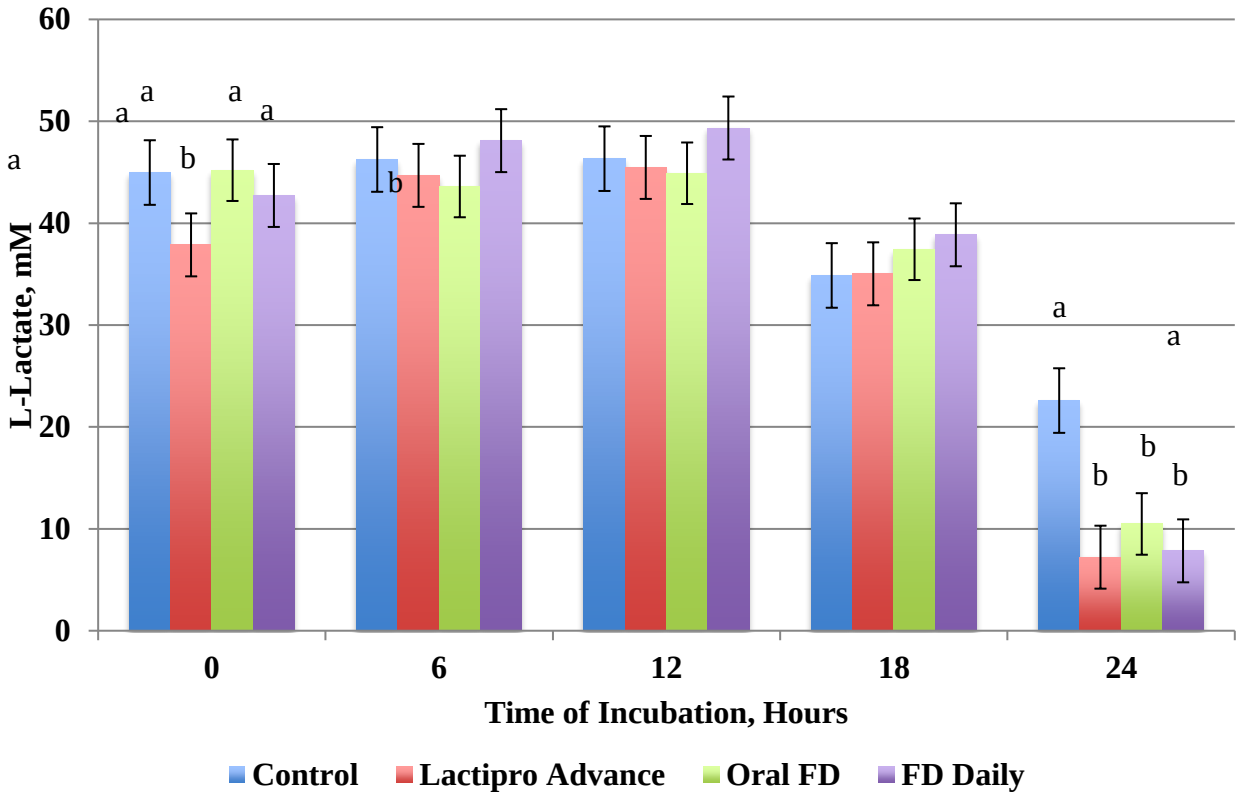
† Control received no *M. elsdenii* and utilized a 21-d step-up; Fresh received an upfront fresh oral drench of Lactipro *advance* (MS Biotec, Wamego, KS) with a 9-d step-up; Rehyd, received an upfront freeze-dried, rehydrated oral drench with a 9-d step-up; Rehyd+Daily upfront freeze-dried, rehydrated oral drench with daily in feed administration and utilized a 9-d step-up

[1]Treatment x time interaction, $P < 0.02$

[2]Effect of treatment, $P < 0.01$

[3]Effect of time, $P < 0.01$

Figure 2.6 Changes in L-lactate concentration of a semi-defined lactate medium inoculated with mixed ruminal microbes



† Control received no *M. elsdenii* and utilized a 21-d step-up; Fresh received an upfront fresh oral drench of Lactipro *advance* (MS Biotec, Wamego, KS) with a 9-d step-up; Rehyd, received an upfront freeze-dried, rehydrated oral drench with a 9-d step-up; Rehyd+Daily upfront freeze-dried, rehydrated oral drench with daily in feed administration and utilized a 9-d step-up

[a,b,c] Bars with different labels are different, $P < 0.05$

Chapter 3 - Literature Cited

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