Effect of Oral Administration of *Megasphaera elsdenii* on Performance of Holstein Cows During Early Lactation

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in
the Graduate School of The Ohio State University

By

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2013

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Abstract

Transition cows have a high risk for metabolic disease within the first 30 days in milk (DIM). Administration of direct-fed microbials (DFM) may reduce the adverse impacts that fresh cows experience during this transition period. *Megasphaera elsdenii* is a lactic acid-utilizing bacterium that has shown promise as a potential DFM during the transition period. Primi- and multiparous Holstein cows (n=162) at a commercial dairy herd were assigned to 1 of 4 treatments: 1) control (no dose) (n=44), 2) 200 mL Lactipro (*M. elsdenii*, 1×10^8 cfu/mL; MSBiotec, Littleton, CO) when cows entered (~ -14 DIM) the close-up pen (n=45), 3) 200 mL Lactipro at 1-3 d postpartum (n=39), and 4) 200 mL Lactipro prepartum and 200 mL at 1-3 d postpartum (n=34). Close-up cows were fed 72% forage (63:37 corn silage and wheat straw), and upon calving, cows entered the high group and were fed 40% forage (90:10 corn silage:wheat straw). The BCS was similar among treatments and no treatment by time interaction occurred. There was no difference in milk yield among treatments ($39.7, 39.7, 39.5$, and 39.8 ± 0.97 kg/d) or peak milk yield. Treatment by parity interactions were detected ($P < 0.05$) for milk yield and peak milk yield in which mature cows (≥ 3 lactations) dosed prepartum had higher daily milk yield and peak milk than the control and pre- and postpartum dosed cows but did not differ from the postpartum dosed cows. The test-day milk fat and protein percentages were similar among treatments. Test-day milk fat was higher ($P < 0.01$) for the first (3.88%) versus second (3.38%) test-day but it was within expected levels, which is

reflective of the low incidence of ketosis in the study. There was a treatment by parity interaction ($P < 0.01$) for milk protein. Milk protein percentage was higher for 2nd lactation cows dosed prepartum than control or cows dosed pre- and postpartum but did not differ from cows dosed postpartum. Control and pre- and postpartum dosed cows with ≥ 3 lactations had higher milk protein percentages than those dosed only prepartum but were not different from cows dosed only postpartum. The SCC did not differ ($P > 0.10$) by treatment or DHI test-day and averaged $333 (x10^3)$ cells/ml across all treatments. Based on urine ketones, only 2.5% of the cows in the study experienced clinical ketosis, but cows with ≥ 15 mg/dL urine ketones were 8.33, 14.3, 14.3, and 0% for treatments, respectively. Health incidences were low in this study and did not differ among the treatments. Reproductive performance also did not differ among the treatments. Based on this study, dosing with *M. elsdenii* prepartum may improve ruminal conditions of high-producing (higher parity) transition cows whereby milk yield is increased. Concentrations of dietary starch and NDF fed both prepartum and postpartum may affect animal response to dosing with *M. elsdenii*.

Dedication

Dedicated to my parents, Tony and Marilyn Stevens, and my brother, Christopher Stevens, for always supporting me in all endeavors I undertake and for constantly showing an interest in my life.

Acknowledgements

Dr. Maurice Eastridge, it seems only fitting that your name appear first in this section as you are the main reason I decided to earn a graduate degree in my year off before veterinary school. You have been my mentor for the past two years and I am grateful to have had the opportunity to work with you on so many research projects. You have not only been my advisor, you have been the one person I know I can always seek guidance from and will challenge me. Second, I would like to thank both Dr. Kristy Daniels and Dr. Jeff Firkins for willingly agreeing to serve on my committee and for always having helpful suggestions when I needed them.

To the interns and fellow graduate students whom have all helped me with this project in some form, I cannot express my gratitude to you enough. Shirron LeShure, you made the early morning trips down to the dairy on sampling days absolutely unforgettable, and I could not imagine trying to get all the milk samples by myself and make it to seminar on time. Sarah Finney, thank you for spending your Tuesday mornings with me collecting blood and urine samples before having to rush off to your afternoon classes, sometimes still smelling like cow. Danni Ye, thanks for helping me figure out the blood assay kits and for always answering my questions. To Jessica Pempek and Benjamin Wenner, you two have probably been two of my best friends this past year and I am so glad I had the opportunity to work alongside you, even though you make fun of me often. To Josie Plank, I cannot thank you enough for your instruction in the lab and

all of the advice you have given me both scientific and personal; you are a truly wonderful person. Thank you to Jill Stiverson, even though I do not work in your lab; you assisted me in using your equipment and were always very helpful with any requests I had. To everyone I have not mentioned, you have all made your mark in my life and I am glad to have gotten to know you this past year.

I would like to thank MSBiotech[®] for their funding of this project and for providing us with the Lactipro product. To the Miedema Family, especially Andy Miedema, this project would not have been possible without your willingness to let me do this project at your dairy farm. Thank you for allowing me access to your production records and for providing me with any data I requested. I would also like to thank the herd manager, Tony, for being patient with me in the chaos at the beginning of the project and for dosing the cows after calving and for helping me get stubborn cows into headlocks.

Last, but certainly not least, I would like to thank all of my lovely black and white ladies of the Miedema Dairy. It was amazing how quickly I fell in love with each of you and memorized all of your different personalities. But not for the dairy cows, none of this would have been possible. Thank you mamas!

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CHAPTER 1: INTRODUCTION

The transition from a dry cow diet to a lactating cow diet is most important in the first 30 days in milk (DIM) as the rumen microbes adjust from a predominantly hay diet to a diet consisting of $\geq 45\%$ concentrates (Counotte et al., 1983). Due to the change in diet and increase in energy requirement for fresh cows, there is a high risk for metabolic conditions, such as ketosis, sub-acute rumen acidosis (SARA), and displaced abomasum within the first 30 DIM. In an attempt to decrease the incidence of these unfavorable metabolic diseases, producers have traditionally used step-up diet transition strategies, feed additives, and ionophores (Klieve et al., 2003). Generally, the preferred industry practice is to gradually introduce grain to the diet over a few weeks in order for rumen microbes to adapt to the high grain diet; however, with the total mixed ration (TMR) system, this is less practical and producers prefer to minimize the number of TMR used. Direct-fed microbials (DFM) are a novel option for decreasing the occurrence of metabolic conditions during the transition period and postpartum. In recent years, their use has increased in part due to the unfavorable view of antibiotic-use by the general public (Martin and Nisbet, 1992; Zebeli et al., 2012). Dosing of DFM holds promise for decreasing the amount of time ruminants spend transitioning from a high forage diet to a low forage diet (Klieve et al., 2003).

Megasphaera elsdenii is a promising DFM in production practices due to its ease of establishment in the rumen and its lactic-acid utilizing capabilities (Klieve et al., 2003; Henning et al., 2010a). The lactic acid-utilization of *M. elsdenii* has the capability of decreasing the risk for lactic acidosis and stabilizing rumen pH in transition cows. In previous dairy cattle studies, lactating cows were dosed postpartum, and none of the studies investigated dosing with *M. elsdenii* prepartum (Hagg et al., 2010; Aikman et al., 2011; Henning et al., 2011; Zebeli et al., 2012). Establishing a viable *M. elsdenii* population before calving and during the transition to a lactating diet could improve animal performance and decrease metabolic risks postpartum.

The risk of metabolic disease in fresh cows is not only detrimental to cow health, but financial implications occur in the cost of treatment and loss in milk production. In addition, as the cost of concentrates increases, feed efficiency in dairy cows becomes increasingly more important. Dosing fresh cows with *M. elsdenii* provides an opportunity to minimize adverse effects of rapidly transitioning from a high forage diet to a high concentrate diet and increase overall lactation performance. In this thesis, performance is defined by: reduced prevalence of metabolic diseases, decreased number of days open, and increased milk production. Improved performance ultimately results in increased producer profitability.

CHAPTER 2: LITERATURE REVIEW

I. Transition Cows

The transition period in dairy cattle is very important because it determines the success of the following lactation. The transition period is described as the 3 wk prepartum through the 3 wk postpartum and encompasses both a change in diet and an increase in energy demand for the newly lactating cow (Grummer, 1995). The 3 wk postpartum are also associated with a 30 to 35% decrease in feed intake in both heifers and mature cows (Grummer, 1995). During this period, the combination of an increase in energy demand for milk production and lowered feed intake causes a negative energy balance (NEB) in the animal. This NEB is most severe in the first week following calving and leads to the rapid mobilization of body tissues to compensate for the energy imbalance.

“Production diseases” can arise as a result of this NEB. Some examples of “production diseases” include metabolic disorders, such as ketosis, hypocalcemia, and hypomagnesemia, as well as displaced abomasum, retained placenta, and laminitis (Mulligan and Doherty, 2008). Other factors such as facilities, grouping strategy, and heat stress also can increase the risk for developing production disease postpartum (Mulligan and Doherty, 2008). In addition, inter-relationships between metabolic diseases can increase the risk for other diseases, including infectious diseases, increased losses in milk

yield, increased lameness, and increased the risk for intramammary infection (Mulligan and Doherty, 2008). Impaired reproductive performance, such as delayed ovulation and lowered conception, also is associated with increased metabolism of body tissue stores, a large decrease in body condition score (BCS), and NEB in the first month postpartum (Butler and Smith, 1989; Jorritsma et al., 2003). Along with NEB, periparturient cows can develop sub-acute rumen acidosis (SARA) due to the abrupt change to a high-grain lactating diet (Enemark, 2009).

Production diseases associated with transition cows are costly to dairy producers due to milk production losses, premature culling, veterinary care, decreased feed efficiency, and increased death loss. Economic losses associated with SARA alone cost between \$500 million and \$1 billion annually (Enemark, 2009). Proper management of transition cows can, therefore, increase the profitability of dairy producers.

II. Feeding the Transition Cow

A. Rumen Microbial Population

The microbial population of the rumen consists of 4 major microbe domains: archaea, bacteria, protozoa, and fungi. These microbes are responsible for the conversion of feedstuffs into absorbable nutrients such as volatile fatty acids (VFA) and, therefore, have a significant role in animal performance. The 3 main groupings of bacteria in the rumen are proteolytic, cellulolytic, and amylolytic. The rumen microbial community varies from animal to animal (Warner et al., 1962; Mohammed et al., 2012). A number of factors can alter the rumen bacterial population, such as the amount of time between

feedings (Warner, 1962), fluctuations in rumen pH (Russell and Dombrowski, 1980), and the concentration of fermentation end products (Hiltner and Dehority, 1983). Changes in the diet, such as a decrease in the forage-to-concentrate ratio, also can shift the rumen bacterial community (Tajima et al., 2000; Tajima et al., 2001; Fernando et al., 2010).

Effect of Changing to a High-Grain Diet

A change in diet from a low concentrate:high forage dry cow diet to a high concentrate:low forage lactating cow diet is essential to provide the energy needed for lactation. A concern with periparturient cows transitioning to a high grain diet is the decrease in rumen pH associated with this type of dietary switch. This drop in pH is due to the rapid fermentation of soluble carbohydrates and resultant accumulation of VFA and lactic acid in the rumen. Cellulolytic bacteria (e.g., *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*) numbers are especially sensitive to low pH, and *F. succinogenes*, *R. albus*, and *R. flavefaciens* will wash out at pH of 6.0, 5.9, and 6.2, respectively (Russell and Dombrowski, 1980). As a consequence of the pH susceptibility of cellulolytic bacteria, cellulose digestion is reduced when pH levels are low (Hiltner and Dehority, 1983). While the activity of cellulolytic bacteria decreases during animal adaptation to a high-grain diet, an increase in amylolytic and lactic acid-utilizing bacteria (*Megasphaera elsdenii*, *Streptococcus bovis*, *Selenomonas ruminantium*, and *Prevotella bryantii*) activity has been shown to occur (Fernando et al., 2010).

B. Rumen Fermentation

Carbohydrates comprise between 60 to 70% of dairy cattle diets and serve as the main energy source for rumen microbes (NRC, 2001). Non-structural carbohydrates (NSC), starches, sugars, organic acids, and fructans are included at greater proportions in lactating cow diets than in dry cow diets in order to meet the higher energy demands of the lactating cow. Increasing the proportion of NSC can decrease fiber digestion and dry matter intake (DMI) (NRC, 2001). In addition, increasing NSC replaces NDF in the diet and decreases rumination, saliva production, and VFA production (NRC, 2001).

The particle size of the diet also can affect rumen fermentation. Beauchemin et al. (2003) observed a reduction in the amount of time rumen pH was ≤ 5.8 when chopped hay was fed compared to ground hay. Particle size also can affect the rate of passage of digesta from the rumen, thus affecting DMI due to rumen fill and rumen pH due to selective retention of coarse particles (Van Soest, 1994; Allen, 1996; Gressley et al., 2011; Kammes and Allen, 2012). Grain processing, such as steam-rolling or flaking, and dietary inclusion of more rapidly fermentable sources of NSC, such as wheat, also can decrease rumen pH (Krause and Oetzel, 2006).

Fermentation Products

Degradation and fermentation of carbohydrates, in conjunction with nitrogen sources, by rumen microbes provides the microbes with the adenosine triphosphate (ATP) needed for cell growth and production of VFA and lactate. The VFA are the primary energy source for the ruminant animal. The 3 main VFA produced by microbial fermentation are acetate, propionate, and butyrate. Acetate is converted to acetyl-coA and

used in the trichloroacetic (TCA) cycle or for fatty acid (FA) synthesis in the mammary gland or adipose tissue. Propionate is a major glucose precursor and is converted to glucose in the liver during the process of gluconeogenesis. Butyrate is the preferred energy source of rumen epithelial cells, can be converted to β -OH butyrate by the rumen epithelium, and also is taken up by the mammary gland for milk fat. Lactic acid is produced by lactic acid-producing bacteria in 1 of 2 isomers: L(+) lactate and D(-) lactate (~ 50:50) (Nagaraja and Titgemeyer, 2007). An accumulation of these fermentation end products, especially D-lactate, may cause a depression in rumen pH, if their production exceeds absorption.

Rumen pH

The typical rumen pH of grain-adapted cattle ranges from 5.8 to 6.5, and a pH of 6.0 to 6.5 is considered ideal (Nagaraja and Titgemeyer, 2007). Rumen pH is affected by a number of feeding conditions, including the type and amount fermentable carbohydrate intake, the buffering capacity of the animal, and rate of utilization and absorption of VFA and lactic acid (Nagaraja and Titgemeyer, 2007). Grain processing, type of grain, particle size, amount and source of NDF, slug feeding of grain, TMR sorting, and DM of the feed can all alter rumen pH by increasing acid production or by decreasing acid removal and buffering capacity.

Increased production of VFA and lactate can depress rumen pH (Owens et al., 1998). Lactate decreases rumen pH more rapidly than VFA and other rumen acids due to its lower pKa and is especially important in lactic acidosis (Owens et al., 1998). The total ruminal VFA and molar proportions of VFA are altered by transition to a high-grain diet.

Both the total molar amount of VFA and the molar proportion of propionate tend to be greater with feeding high-grain versus high-forage diets (Tajima et al., 2000; Penner et al., 2009). The molar proportion of acetate and the acetate-to-propionate ratio are lower in animals fed high-grain diets (Penner et al., 2009). The increase in VFA production increases the rate of absorption of VFA across the rumen epithelium, due to the concentration-dependency of VFA absorption. The VFA are passively absorbed across the rumen epithelium in the protonated form, and as a result, a decrease in rumen pH also increases the rate of absorption (Dijkstra et al., 1993; Owens et al., 1998). Feeding a highly fermentable diet also has been shown to increase the expression of VFA anion transporters, and the increase in transporter expression occurs before the morphological changes in rumen papillae (Penner et al., 2011). The increase in VFA associated with the transition from high forage to a high grain diet promotes the growth of rumen papillae (Kleen et al., 2003). Total elongation of rumen papillae may take 4 to 6 wk; if the shift from the high forage diet to the high grain diet is too abrupt, the papillae may not have enough time to increase in surface area to allow for optimal VFA absorption (Hutjens, 1996).

C. Ruminal Acidosis

Ruminal acidosis is classified as either sub-acute (SARA) or acute. The SARA and acute acidosis are defined as a depression in rumen pH below 5.6 and 5.2, respectively (Owens et al., 1998). This drop in pH with acute acidosis is due to an accumulation of lactic acid in the rumen (Nagaraja and Titgemeyer, 2007). However, SARA is described as a depression in ruminal pH due to VFA accumulation, with very

little lactate detected in the rumen (Krause and Oetzel, 2006; Nocek, 1997). The SARA is regarded as more economically important than acute acidosis because SARA-affected animals may not appear sick but have decreased feed intake and production (Slyter, 1976; Owens et al., 1998).

Rumen Microorganism Changes Associated with Acidosis

In order to treat and ultimately prevent the incidence of ruminal acidosis, the cause of the accumulation of organic acid in the rumen and resultant pH depression must be identified. In dairy cattle, the adaptation to a high-concentration diet around calving increases the availability of readily fermentable carbohydrates in the rumen. This increase in starch concentration promotes amylolytic microbe activity, such as that of *Streptococcus bovis* and *Selenomonas ruminantium* (Nagaraja and Titgemeyer, 2007). *S. ruminantium*, *S. bovis*, and *Lactobacillus* spp. have both fast growth rates and the ability to rapidly degrade starch and soluble sugars, which results in the accumulation of VFA and lactic acid (Nagaraja and Titgemeyer, 2007). Goad et al. (1998) observed a 10-fold increase in ruminal *Lactobacillus* numbers in grain-adapted compared to hay-adapted steers, and Fernando et al. (2010) observed a 2-fold increase in *S. bovis* at the beginning of the step-up regimen and a 30-fold increase in *S. ruminantium* during the entire adaptation period to a high-grain diet.

Streptococcus bovis is a major lactate-producer and is relatively tolerant of low rumen pH (Russell and Hino, 1985). In the presence of excessive substrate, *S. bovis* proliferates rapidly and produces lactic acid (Russell et al., 1981). Lactate production in *S. bovis* is fructose 1,6-disphosphate dependent, and lactate dehydrogenase is feed-

forward activated by fructose 1,6-diphosphate (Bond and Russell, 1996). The increase in lactic acid production as the pH declines further promotes acidosis and will eventually lead to acute acidosis (Russell and Hino, 1985; Asanuma et al., 1997; Nagaraja and Titgemeyer, 2007). *S. ruminantium* both produces and utilizes lactic acid (Nagaraja and Titgemeyer, 2007). Strains of *S. ruminantium* that utilize lactate are classified as spp. *lactilytica*, while the strains that do not utilize lactate are grouped in the subspecies *ruminantium* (Nagaraja and Titgemeyer, 2007). *S. ruminantium* spp. *ruminantium* is the predominant organism, whereas spp. *lactilytica* accounts for less than 1% of the total *S. ruminantium* population (Yoshii et al., 2003). As rumen pH falls, most ruminal bacteria are inhibited and the acid-tolerant anaerobic *Lactobacilli* predominate (Slyter, 1976; Nagaraja and Titgemeyer, 2007). In addition to the lactic acid-producing bacteria, holotrich protozoa also are capable of producing lactate (Morgavi et al., 1994).

Rumen microorganisms, such as *M. elsdenii*, *P. shermanii*, and *S. ruminantium* spp. *lactilytica*, utilize lactic acid and help to stabilize the rumen pH (Nocek, 1997). These organisms are responsible for the further metabolism of the fermentation intermediate, lactate, to VFA (Nagaraja and Titgemeyer, 2007). Based on cultured bacteria, *M. elsdenii* is the major lactate utilizer and has a vital role in the prevention of lactate accumulation in the rumen, as *M. elsdenii* utilizes 60 to 80% of the lactate produced in the rumen of cattle (Counotte et al., 1981). Both the L- and D- forms of lactate can be used by *M. elsdenii*, and it is the only characterized rumen microorganism known to ferment lactate to propionate via the acrylate pathway (Counotte et. al., 1981). *S. ruminantium* spp. *lactilytica* utilizes less lactate and metabolizes only D-lactate, as it possesses little to no lactate racemase or L-lactate dehydrogenase (Asanuma and Hino,

2005). Bacteria are not the only rumen microorganisms that utilize lactic acid; entodiniomorphid protozoa also are capable of using lactic acid (Newbold et al., 1987; Morgavi et al., 1994). The entodiniomorphid protozoa produce propionate and butyrate from lactate (mostly L-lactate) (Morgavi et al., 1994).

D. Prevention of Acidosis

The ultimate goal in any business is to prevent problems before they occur. The dairy industry benefits from feeding management practices that aim to prevent metabolic diseases, such as acidosis. Acidosis is especially important to prevent as it affects feed intake, rumen fermentation products, rumen microflora, milk production, and can increase the risk for diarrhea, laminitis, and liver abscesses (as cited in Plaizier et al., 2009). The traditional management practice to prevent ruminal acidosis is to gradually introduce dairy cattle to a higher concentrate diet; however, grouping strategies (limiting number of groups and group moves) and feeding management practices (e.g., TMR usage) limit using this approach today (Meissner et al., 2010). Controlling acidosis via a method other than a feed-adaptation period would likely be more economical because less forage would be fed and an earlier increase in concentrate might boost milk production.

Buffers

Feed, ammonia, and saliva can all function as ruminal buffers by resisting a change in rumen pH (Allen, 1997). The buffering capacity of ingested feed produced in

the rumen is optimal when the rumen pH is low, but negligible at normal rumen pH (Allen, 1997). Ammonia produced from protein degradation has some buffering capacity, but it is rapidly absorbed through the rumen epithelium (Allen, 1997). Saliva production is directly correlated with the amount of forage in the diet, and saliva flow and buffering capacity is greatly decreased when forage content is low (< 30% forage) (Erdman, 1988).

The inclusion of dietary buffers, such as bicarbonate salts, is a regular practice in both feedlot and dairy cattle nutrition, in which low forage diets are fed to increase gain or milk production. Adequate dietary buffers should be water-soluble and have a pKa value similar to the physiological pH of the rumen (Enemark, 2009). The palatability and effectiveness of mixing a buffer into a ration should also be considered because this could decrease feed intake (Erdman, 1988). The NRC (2001) suggests that dietary buffers be fed at 0.6 to 0.8% of DMI and will benefit early lactation cows fed a high concentrate diet the most.

Feeding Management

In order to achieve optimal milk production and lessen NEB, early lactation diets are formulated with a greater proportion of NFC and less fiber. Motivation for reducing the percentage of NDF is to increase digestibility and DMI. The NDF is, on average, less digestible than NFC and can negatively affect DMI because of rumen fill (NRC, 2001). Increasing dietary NDF, forage NDF (fNDF), and the amount of digestible organic matter (OM) of forages positively influences ruminal pH (Zebeli et al., 2006). Firkins et al. (2001) suggested that, in addition to ruminal pH, chewing time and the acetate-to-propionate ratio also are correlated with the dietary fNDF-to-NSC ratio. Reducing the

percentage of fiber can have an adverse affect on rumen health and ruminal pH, due to a reduction in buffering capacity (ie. chewing and saliva production) (NRC, 2001; Zebeli et al., 2006). Therefore, decreasing the NDF fraction predisposes early lactating cows to SARA. The NRC (2001) recommendations for NDF are 25% of dietary DM and 19% of the total dietary DM from fNDF when the NFC is 44% of dietary DM. The source and physical form of the fiber and feed sorting by cows should be evaluated and considered when adjusting actual dietary recommendations of NDF and fNDF.

Both the chemical characteristics (i.e., NDF) and the physical characteristics of the fiber are important to consider when the forage-to-concentrate ratio is decreased (Mertens, 1997). The particle size and density of the fiber source can affect ruminal fermentation, animal health, and milk fat yield regardless of the NDF amount (Mertens, 1997). The ability of fiber to influence chewing activity and the density of the ruminal fiber mat is defined as physically effective NDF (peNDF) and is measured as the product of NDF content and the physically effective factor (Mertens, 1997). The effects of peNDF on ruminal pH, chewing activity, and fiber digestibility appear to be inconsistent and variability between techniques used to measure peNDF creates difficulty in establishing dietary requirements for peNDF (Beauchemin et al., 2003; Yang and Beauchemin, 2006; Zebeli et al., 2006). The suggested amount of peNDF is 19 to 22% of DMI based on the particle retention by a 1.18 mm screen to maintain an average ruminal pH of 6.0 (Beauchemin et al., 2003; Zebeli et al., 2006). The variability of peNDF in nonforage fiber sources should be considered when they are included at high levels in dairy cow diets because they are less effective at stimulating chewing and maintaining ruminal pH (Allen, 1997).

Environmental Stress (Heat Stress and Grouping)

Environmental conditions can influence the incidence or severity of ruminal acidosis by affecting feed intake and behavior. Heat stress and facility conditions such as stall comfort, overcrowding, and bunk management can all alter cow behavior and negatively impact cow health (Stone, 2004). Grouping strategy can affect DMI due to cow comfort and competition for feed and water (Grant and Albright, 2001). Konggaard and Krohn (1978) (as cited in Grant and Albright, 2001) noted an increase in DMI and milk production by primiparous cows when they were housed separately from multiparous cows, likely due to less competition at the feed bunk and decreased stress. Decreasing feed bunk space increases feeding competition and reduces feeding time and intake (Friend et al., 1977; Grant and Albright, 2001). Heat stress depresses feed intake by altering cow behavior and meal patterns (Sniffen et al., 1993). Altered feeding patterns caused by thermal stress include “slug-feeding” (Mallonée et al., 1985), reduced rumination (Collier et al., 1982), and decreased DMI (McGuire et al., 1989; Lough et al., 1990). The DMI of thermal stressed cows is reported to be 3 to 4 kg/d lower than that of thermal comfort cows (Lough et al., 1990; McGuire et al., 1989). The reduction in DMI associated with an increase in ambient temperature is important to consider for early lactation cows that already have depressed intakes and are at elevated risk for metabolic disease (Grant and Albright, 1995). In the study by Mishra et al. (1970), when cows were subjected to the “cool” condition of 18.3°C and 50% humidity compared to the “hot” condition of 29.4°C and 85% humidity, mean rumen pH declined from 6.3 to 5.9, respectively. A reduction in the time spent lying in free stalls also is associated with an increase in ambient temperature (Overton et al., 2002). Due to the alterations in feeding

behavior by heat stress, increasing the peNDF and decreasing NSC during the summer may help reduce the risk of SARA (Stone, 2004). Concurrently, replacement of some NSC with fat supplementation will increase the energy density of the diet when DMI is depressed during heat stress (Wang et al., 2010). The feeding of yeast culture during periods of heat stress also may improve feed efficiency and milk production (Schingoethe et al., 2004; Bruno et al., 2009). Proper environment and facility management, in addition to nutrition, are important to minimize the incidence of metabolic disease, particularly SARA (Stone, 2004).

Ionophores

Ionophores are a type of chelating agent that bind monovalent ions, such as sodium and potassium (Van Soest, 1994). The primary function of ionophores is to alter rumen bacterial populations by inhibiting the growth of some ruminal bacteria, such as *S. bovis* and *Lactobacillus spp.* (Dennis and Nagaraja, 1981; Chow and Russell, 1990). Two commonly used ionophores in dairy cattle rations are monensin and lasalocid. Monensin and lasalocid penetrate the cell membrane bacteria and cause a disruption in the efflux of ions (Russell, 1987). Ionophores are proposed to prevent or decrease the severity of ruminal acidosis by inhibiting lactate-producing bacteria, thereby reducing lactate production (Enemark, 2009). The results of monensin supplementation in studies using lactating cows vary. In a lactating cow study, Osborne et al. (2004) observed a 19.7% increase in total tract fiber digestibility with monensin supplementation (22 mg of monensin/kg of TMR), but monensin had no effect on DMI, milk yield, or milk

composition. Dietary monensin inclusion does not appear to significantly affect ruminal pH characteristics during SARA conditions in dairy cows (Mutsvangwa et al., 2002; Osborne et al., 2004).

The function of ionophores in altering rumen fermentation characteristics is inconsistent. Sauer et al. (1989) observed an increase in the molar proportions of propionate and valerate and a decrease in acetate and butyrate with the addition of monensin to the diet of lactating dairy cows. A similar increase in propionate with monensin addition was observed during in vitro incubations with corn (Russell and Strobel, 1988). In contrary, the studies performed by Oelker et al. (2009), Mathew et al. (2011), and Reveneau et al. (2012) did not observe an increase in the propionate or a decrease in acetate and butyrate with the addition of monensin. The decreased plasma β -hydroxybutyrate (BHBA) concentrations in postpartum cows fed a monensin-containing ration suggest monensin supplementation improved energy balance (Sauer et al., 1989; Mullins et al., 2012). Despite a decrease in plasma BHBA concentrations in monensin-supplemented cows, milk production was not significantly improved (Sauer et al., 1989; Mullins et al., 2012). The potential of ionophores to increase the performance of lactating dairy cows is most likely related to increased feed efficiency than a change in the acetate:propionate ratio (Ipharraguerre and Clark, 2003). The benefits of ionophore usage in dairy cattle may also vary depending on dietary characteristics, environmental conditions, and production level of cows.

Direct-Fed Microbials

The DFM are live, naturally occurring microorganisms, such as yeast, fungi, and bacteria, which beneficially alter microorganism populations (Yoon and Stern, 1995; Ghorbani et al., 2002). Interest in the effect of DFM on animal performance has increased as concern of antibiotic-use in the food-animal industry has grown (Martin and Nisbet, 1992). Although the definite mechanisms of action by DFM have not been established, there are some suggested modes. Yoon and Stern (1995) suggested that DFM function by modifying microbial populations of the rumen or lower gut, altering rumen microbial fermentation, increasing the intestinal nutrient flow, improving diet digestibility, and enhancing host immunity. Lactate-producing bacteria, such as *Lactobacillus*, have been used in commercially available DFM products (Kung, 1999), but variable animal responses have occurred (Yoon and Stern, 1995). The concept of using lactate-producing bacteria is to adapt the ruminal microbe populations to the presence of lactic acid (Ghorbani et al., 2002). The use of lactate-utilizing bacteria, such as *M. elsdenii*, is to prevent the accumulation of lactic acid (Kung and Hession, 1995). Ghorbani et al. (2002) observed an increase in acetate concentration and an indication of decreased incidence of acidosis in feedlot cattle with supplementation of a combination of *Propionibacterium* and *Enterococcus*.

Certain yeasts have been shown to benefit cellulolysis and growth of cellulolytic bacteria. Williams et al. (1991) reported an increase in milk yield with dietary inclusion of *Saccharomyces cerevisiae*. There is some uncertainty of the viability and ability of yeasts to multiply in the rumen due to anaerobiosis (Brown and Nagaraja, 2009).

Inclusion of fungal cultures may improve fiber digestibility and increase rumen pH by stimulating the growth of cellulolytic and lactate-utilizing rumen bacteria (Brown and Nagaraja, 2009). *Aspergillus oryzae* and *S. cerevisia* have been shown to decrease lactic acid accumulation by stimulating lactate utilization by *S. ruminantium* in pure culture (Martin and Nisbet, 1992). Overall, production results with fungal DFM and yeasts have been inconsistent (Beauchemin et al., 2000; Brown and Nagaraja, 2009).

Many DFM are commercially available that hold promise for enhancing animal performance. Supplementation of DFM is typically most beneficial to animals under stress or with lowered immune responses, such as early lactation cows, high producing cows, or clinically ill cows that have been given antibiotics (Beauchemin et al., 2000; Krehbiel et al., 2003; Nocek et al., 2003). In addition, DFM may enhance fiber digestibility; which is especially important in the summer when fiber digestibility can be compromised because of heat stress (Beauchemin et al., 2003). However, the use of DFM as a means to alter ruminal microbial fermentation has proven to be variable. Further research into the activity of DFM and the optimal conditions at which they operate is needed (Yoon and Stern, 1995; Krehbiel et al., 2003; Brown and Nagaraja, 2009).

III. *Megasphaera elsdenii*

Megasphaera elsdenii is an obligate anaerobe bacterium found in the rumen of ruminant animals. *M. elsdenii* is a predominant lactate-utilizer and is dependent on the products of polymer hydrolysis, such as soluble sugars and lactate, performed by other rumen microbes (Marounnek et al., 1989). *M. elsdenii* grows well in vitro on a variety of substrates (glucose, fructose, maltose, glucosamine, mannitol, lactate, and pyruvate) and

produces different end products from glucose (predominantly butyrate) than lactate, and end products are variable by strain (Marounek et al., 1989). *Megasphaera elsdenii* differs from other lactate-utilizing bacteria because it lacks the catabolite repression by glucose and maltose that hinders lactate utilization in similar organisms (Hino and Kuroda, 1993). The ability of *M. elsdenii* to preferentially utilize lactate has peaked interest in using this organism as a DFM for grain-fed cattle.

A. Metabolism of Lactate by *M. elsdenii*

Over 60% of the lactate produced in the rumen is metabolized by *M. elsdenii* and includes both the L- and D- forms of lactate (Counotte et al., 1981). *Megasphaera elsdenii* possesses 2 possible pathways for fermenting lactate and these pathways are based upon the isoform of lactate (D- or L-) present. The conversion of L- lactate to D- lactate by lactate racemase (LR) is readily reversible and activity of LR is inducible by the presence of lactate (Hino and Kuroda, 1993; Hino et al., 1994). D-lactate is constitutively converted to pyruvate via NAD-independent D-lactate dehydrogenase (iD-LDH) (Hino and Kuroda, 1993; Hino et al., 1994). The end products of D-lactate fermentation are butyrate and acetate via acetyl-CoA.

The L-lactate is converted to propionate via the acrylate pathway with acrylyl-CoA as an intermediate (Hino and Kuroda, 1993). *Megasphaera elsdenii* is the only known rumen microbe to use this pathway to convert lactate to propionate (Nagaraja and Titgemeyer, 2007). Of the amount of lactate fermented, over 70% is converted to propionate via the acrylate pathway (Counotte et al., 1981).

The directionality of lactate through the 2 lactate-utilization pathways is both concentrate- and pH-dependent. In batch culture, where lactate is present in excess, *M. elsdenii* principally produced propionate, along with acetate and butyrate. Under carbon-limited conditions, in which minimal lactate was present, acetate and butyrate were formed but not propionate (Prabhu et al., 2012). The lack of sufficient lactate flux to drive the formation of lactyl-CoA from L-lactate and subsequent formation of acrylyl-CoA explains why *M. elsdenii* grown on only glucose failed to produce propionate from the acrylate pathway (Counotte et al., 1981; Prabhu et al., 2012). In contrast, the reaction of D-lactate to pyruvate via iD-LDH is favored when lactate concentration is low, and acetate and butyrate are the principal products (Hino et al., 1994). *M. elsdenii* will preferentially ferment lactate until low concentrations, 1 to 2 mM, before it utilizes glucose as a substrate (Hino et al., 1994). Counotte et al. (1981) observed that as rumen pH decreases, more butyric acid is fermented from DL-lactate; therefore, the fermentation of DL-lactate to butyrate is pH-dependent (most produced at pH 5.25 to 6.0).

An increase in the ruminal *M. elsdenii* population during the transition period in dairy cattle has the potential to not only prevent acidosis but also improve energy balance by converting the excess lactate to propionate. The increase in lactate production by *S. bovis* and *Lactobacillus spp.* associated with the transition to a high-grain diet may provide the lactate flux necessary for the production of propionate via the acrylate pathway (Counotte et al., 1981). Microbial propionate production influences the energy balance of transition cows because 80% of the propionate produced in the rumen is converted to glucose in the liver through gluconeogenesis (Allen et al., 2009). The supply

of propionate to the liver is important for transition cows at risk for NEB, ketosis, and fatty liver, and increasing propionate production may reduce the incidence and severity of these metabolic diseases (Wang et al., 2012).

B. In vitro Trials

The lactate fermenting ability of *M. elsdenii* has been studied in cultures for many years. Some strains of *M. elsdenii* have proven better at preventing lactic acid accumulation and an excessive drop in pH than others. The *M. elsdenii* B159 strain studied by Kung and Hession (1995) prevented lactate accumulation and pH drop when incubated in mixed rumen culture with highly fermentable carbohydrates (comprised of 55% starch and 26% glucose). The CH7 and CH4 strains studied by Henning et al. (2010b) were the most efficient in utilizing lactic acid and maintaining lactic acid concentration below 10 mmol/L in comparison to the other 9 strains studied, when incubated in batch cultures of rumen fluid with added maltose as substrate. The amount of time the culture pH stayed < 6.0 was also significantly lower for strains CH7, CH3, and CH4.

The end products of fermentation by *M. elsdenii* also vary by strain and by substrate. Marounek et al. (1989) studied 4 different strains of *M. elsdenii*, and the production of metabolites varied by strain and by amount of glucose added to the lactate medium. In lactate medium, strains LC1 and AW106 produced mostly acetate and propionate; strain L8 produced almost equal amounts of acetate, propionate, and butyrate; and butyrate was the predominate metabolite produced by strain J1. As glucose was added to the lactate medium, the propionate produced by all 4 strains declined and

butyrate production increased. In a study by Kung and Hession (1995), cultures with the B159 strain of *M. elsdenii* that were incubated in ruminal fluid inoculated with substrate containing 55% soluble starch, 26% glucose, 6% cellulose, 7% cellobiose, and 6% trypticase (by weight) had lower concentrations of acetate and propionate and higher concentrations of butyrate than the control. Similarly, Forsberg (1978) observed an absence of detectable levels of propionate when strain B159 was grown in low glucose medium, although acetate, propionate, butyrate, and valerate were produced when grown in low lactate medium. The NIAH 1102 strain of *M. elsdenii* produces mainly acetate and butyrate when grown on glucose only, with minimal propionate production (Hino and Kuroda, 1993; Hino et al., 1994). When this particular strain was grown on either D- or L-lactate, similar quantities of acetate, propionate, and butyrate were produced. In addition, when acrylate was added to either the L-lactate or the glucose substrate, propionate was the predominate metabolite produced (Hino and Kuroda, 1993). When lactate was the sole carbon source strain, YE34 primarily produced butyrate and valerate (Ouwkerk et al., 2002). Horn et al. (2009) reported that strain CH4 (also known as NCIMB 41125) converted lactate to mainly acetate, and the proportions of butyrate, valerate, and propionate produced were dependent on pH and dilution rate.

Of all the strains of *M. elsdenii* studied in vitro, the NCIMB 41125 strain has shown the most promise for use as a DFM to prevent or alleviate ruminal acidosis. It has a high growth rate (0.94/h) and a long survival rate on medium (20 d) (Horn et al., 2009). This biomass accumulation could provide protection from harsh environmental factors, such as oxygen exposure, when used in a commercial application (Meissner et al., 2010). This specific strain is relatively resistant to low pH and is able to produce fermentation

end products at pH below 5.5, even though a sharp decline in growth is observed between pH 5.5 and 6.0 (Horn et al., 2009). The robustness and lactic acid utilization ability of *M. elsdenii* NCIMB 41125 has led to research aimed at evaluating the effects of administering this strain to animals at risk for developing ruminal acidosis.

C. Establishment of *M. elsdenii* in vivo

In order for a DFM to be efficient, the microbe must first be readily established in the rumen. Studies on the establishment of *M. elsdenii* in ruminant animals have shown that this microbe can be successfully established in the rumen. In the Kileve et al. (2003) study with beef steers inoculated via rumen cannula with 5.5×10^{11} cfu of *M. elsdenii*, the rumen population of *M. elsdenii* immediately after inoculation was $>10^6$ cell equivalents (CE) ml⁻¹ and increased throughout the trial to an excess of 10^8 CE ml⁻¹. Prior to inoculation or grain introduction, *M. elsdenii* was not detectable in any of the steers, inoculated or control. The average density of *M. elsdenii* detected in the control steers did not surpass 10^4 CE ml⁻¹ before d 12 but then increased to similar abundance as inoculated cows by d 21 (Klieve et al., 2003). The inoculated population of *M. elsdenii* established 5 to 7 d sooner in the rumen than the wild type population (Klieve et al., 2003). In the study by Henning et al. (2010a), the rumen population of *M. elsdenii* was higher in steers inoculated with 4×10^6 cfu/ml *M. elsdenii* CH4 (NCIMB 41125) than control populations until 5 d after dosing, at which time control populations caught up to the inoculated. Due to the rapid establishment of *M. elsdenii* in the rumen of grain-fed animals, it holds promise for use as a DFM for ruminants transitioning from high forage to high grain diets.

D. Beef Cattle and Sheep

Beef Cattle

Klieve et al. (2003) studied the establishment of *M. elsdenii* and *Butyrivibrio fibrisolvens* in 10 Hereford steers during the transition from a high forage diet to a high-grain end diet of 75% rolled barley. Grain adaptation was implemented in 3 steps; the first 2 days steers received 45% rolled barley followed by 2 days of 60% rolled barley, and finally 75% rolled barley for the remainder of the 22 d experiment. All of the diets were balanced with paspalum hay. Five of the steers were inoculated via rumen cannula with 500 mL of 5.5×10^{11} cfu *M. elsdenii* YE34 and 3×10^{12} cfu *Butyrivibrio fibrisolvens* YE44 and the remaining 5 were control steers. Prior to inoculation or grain introduction, *M. elsdenii* was not detected in any of the steers, but it increased throughout the study. Although *M. elsdenii* was present in both treatment groups, the *M. elsdenii* density in the rumen of uninoculated steers did not reach similar levels to those of the inoculated steers until 21 d after grain introduction. Despite the successful establishment of *M. elsdenii*, there were no differences between the inoculated and control steers in feed intake, rumen pH, or lactic acid concentration. The lactate concentration for all animals did not rise above 2 mmol/L and rumen pH remained above 5.6 for all steers. Although VFA did not differ by *M. elsdenii* inoculation, grain introduction caused a minor decrease in acetate and a doubling of propionate and butyrate concentrations in all animals.

In the study by Henning et al. (2010a), 14 beef steers were administered with either control (no dose), low (1.72×10^9 cfu), medium (1.72×10^{10} cfu), or high (1.72×10^{11} cfu) doses of *M. elsdenii* NCIMB 41125 (CH4) and transitioned to a 94% concentrate diet in a 5 step-up program over 20 d. Rumen *M. elsdenii* counts did not differ between

the 3 *M. elsdenii* dosages. The control steers had lower *M. elsdenii* counts in the first 2 to 3 d after inoculation and, subsequently, had higher lactic acid concentrations. Total VFA concentrations did not differ between inoculated and control steers, but inoculated steers tended to have high butyrate and acetate and lower propionate concentrations. The shift towards more butyrate may be indicative of the rumen pH being near the “propionate to butyrate shift” that occurs at lower pH.

McDaniel et al. (2009) evaluated the effects of administration of *M. elsdenii* in steers faced with an abrupt diet change. The 20 steers were divided into 4 treatment groups and were dosed with either 10, 100, or 1000 ml of 1.62×10^8 cfu/ml of live *M. elsdenii* NCIMB 41125 or a placebo on d 2 of the experimental period. Steers were fed a flaked corn diet consisting of 34% roughage and 66% concentrate *ad libitum*. Although the lactic acid concentration increased and rumen pH declined in all animals following the diet change from 100 to 34% roughage, the lactate concentrations were lower for steers administered *M. elsdenii*, regardless of dosing amount, and the *M. elsdenii*-treated steers maintained higher rumen pH in the 24 h after the diet change than the placebo steers.

Leeuw et al. (2009) studied the effect of dosing *M. elsdenii* on the performance of steers fed low roughage (2%) or high roughage (8%) diets. Half of the steers received a 200-ml dose of 10^9 cfu/ml *M. elsdenii* NCIMB 41125 and half received a 200-ml dose of water. Steers in both of the dosing treatments were further divided in half and received either the low or high roughage diet. Neither differences in average daily intake and overall average daily gain (ADG) nor carcass characteristics differed between treatments. Dosed steers had better performance as indicated by a lower feed-to-gain ratio and higher

ADG in the few weeks immediately post the adaptation period. This improvement in animal performance coincided with a reduced tendency of morbidity, other than diarrhea and bloat. In addition, steers on the low roughage diet had significantly lower incidences of diarrhea and bloat when dosed with *M. elsdenii*.

Drouillard et al. (2012) evaluated the effect of dosing steers with *M. elsdenii* NCIMB 41125 in 2 adaptation periods of varying length. Half of the steers were dosed with 200 ml of inoculum containing 10^{11} cfu *M. elsdenii* cells or a placebo; half of the steers in each of these 2 treatments were enrolled in either an 8- or 17-d transition period, containing 3 or 5 steps, respectively. The initial diets in both transition periods consisted of 45% roughage and 55% concentrate, and the final diets consisted of 6% roughage and 94% concentrate. Feed intake, body weight (BW), and ADG did not differ by treatment. Carcass characteristics also did not differ by treatment; however, there was a trend for greater carcass-adjusted ADG and hot carcass weight in the inoculated steers.

Sheep

In the second part of the Henning et al. (2010a) study, 24 wether lambs were divided into 4 treatment groups in a 2×2 factorial design. The wethers were either drenched with 200 ml of 10^{11} cfu *M. elsdenii* NCIMB 41125 or not drenched and were fed forage *ad libitum* or restricted-fed forage. The amount of concentrate consumed did not differ between the *ad libitum* or restricted-fed forage treatments, but the sheep drenched with *M. elsdenii* consumed 11% more concentrate from d 1 to slaughter than their placebo-drenched counterparts and consumed less forage. Despite the lower forage intake by the drenched wethers, they had less visual signs of diarrhea in the first few

weeks of concentrate feeding, and the decline in rumen pH was less. Lactic acid accumulation in the first 2 to 3 d after also was less for the drenched wethers. Although the concentrate intake was higher in the drenched wethers, there were no differences for ADG or dressing percentage between the control and *M. elsdenii* drenched animals.

Henning et al. (2010b) followed up *in vitro* work with *M. elsdenii* strains CH4 and CH7 in a study with 18 rumen-cannulated sheep. The sheep were administered with a 100-ml dose containing either 10^{11} cfu of CH4 or CH7 prior to grain introduction. Rumen pH in sheep inoculated with the CH4 strain never fell below a pH of 5.5 and recovered to 6.0 by 24 h after the induced grain challenge. Conversely, 24 h after the induced-grain challenge, the CH7 strain remained at 5.5 and the control strain was below 5.0. Lactic acid concentrations were lower for *M. elsdenii* inoculated sheep than control. The lactic acid concentration for both the CH4 and CH7 strains never went above 10 mmol/L, whereas the control lactic acid concentration reached 55 mmol/L in the 12 h post-dosing. Feed intake was greater for sheep inoculated with *M. elsdenii* than for control. The higher feed intake is expected because the pH remained higher and that lactic acid concentrations were lower in the inoculated sheep.

E. Dairy Cattle

The effect of administration of *Megasphaera elsdenii* on dairy cattle production has been studied less extensively than that of the feedlot industry. In a study performed by Hagg et al. (2010), 60 multiparous Holstein cows were randomized based on parity and previous lactation into 1 of 4 treatment groups: low concentrate dosed (LCD), low

concentrate control (LCC), high concentrate dosed (HCD), and high concentrate control (HCC). On d 2, 10, and 20 post-partum, the LCD and HCD cows were orally-dosed with 250 mL of 10^9 cfu/ml of *M. elsdenii*. The mean rumen pH was similar among the *M. elsdenii* and control treatments. The lactic acid concentration was significantly higher in the HCC diet than either of the LCD or LCC treatments, but it did not differ from the high concentrate *M. elsdenii* treatment. No differences for total rumen VFA or individual VFA molar proportions between *M. elsdenii*-dosed animals and control were observed. However, propionate concentration in the rumen was significantly higher in those fed the high-concentrate diets compared to the low concentrate diets. Dosing *M. elsdenii* did not affect yields of milk, protein, and lactose, or concentration of milk urea nitrogen. The fat percentage of cows dosed with *M. elsdenii* and fed a high concentrate diet (70% concentrate) tended to be lower (2.88%) than the non-dosed cows fed the same high concentrate diet (3.60%). Dry matter intake, feed efficiency (kg milk/kg DMI), BW, and BCS did not differ with *M. elsdenii* dosing. Hagg et al. (2010) measured fecal score and pH on 15 and 30 DIM. Fecal score measured on 15 and 30 DIM was unaffected by *M. elsdenii* treatment, but fecal pH ($P < 0.05$) were 6.62 and 6.43 for dosed and control treatments, respectively. The authors suggested that the difference in fecal pH was due to more ruminal fermentation of starch in the dosed versus control cows, which was supported by the lower fecal starch content for the dosed cows.

In the fresh cow study by Aikman et al. (2011), 14 multiparous, rumen-cannulated Holstein cows were enrolled in the study. On d 3 and 12 postpartum, cows were dosed via rumen cannula with either 250 ml of *M. elsdenii* (10^{10} to 10^{13} CFU) or a placebo dose of autoclaved suspension. The mean rumen pH was similar between *M. elsdenii* and

control treatments. However, the length of time that rumen pH was below 5.6 was decreased in the days immediately following dosing with *M. elsdenii*, and pH fluctuated less from day to day in these cows. Despite the reduced time that rumen pH was below 5.6, there was no difference in lactic acid concentration and total rumen VFA concentration by *M. elsdenii*-dosed animals compared to control animals. A treatment by day interaction for the acetate:propionate ratio did occur in which the ratio for placebo cows declined from d 4 to 15 and the *M. elsdenii*-dosed cows declined from d 4 to 6 but then increased from d 13 to 15. Milk production efficiency was greater in dosed cows, almost 0.3 kg/kg of DMI, and was a result of a decrease in DMI and concurrent non-significant increase in milk yield. The milk fat concentration tended to be 5.2 g/kg lower in *M. elsdenii*-dosed cows than placebo cows. This reduction in milk fat could be due to the shift in ruminal fermentation products for *M. elsdenii*-dosed cows, such as a decrease in lipogenic acetate and an increase in gluconeogenic propionate compared to cows receiving placebo. The authors also suggested an abundance of *M. elsdenii* in the rumen could be responsible for the lower milk fat concentration as Palmonari et al. (2010) and Weimer et al. (2010) have demonstrated an inverse relationship between milk fat concentration and *M. elsdenii* abundance. Furthermore, several strains of *M. elsdenii* have been noted to produce *trans*-10,*cis*-12 conjugated linoleic acid in the rumen, which inhibits milk fat synthesis in the mammary gland (Kim et al., 2002). However, the reduction in milk fat may be better explained by a reduction in mobilization of body fat stores, as observed in the a 14-wk companion study by Aikman et al. (2009), in which high-yielding cows dosed with *M. elsdenii* tended to gain more weight and have reduced milk fat concentrations on an acidosis-challenge diet (39:61 forage:concentrate ratio) than

placebo cows on the same diet. The effect of *M. elsdenii*, specifically strain NCIMB 41125, on milk fat concentration is still unclear. Milk protein concentration also was lower in *M. elsdenii*-dosed high yielding cows in the study by Aikman et al. (2009), but the reduction could be a dilution effect caused by the 2.2 kg/d increase in milk yield for cows on the acidosis-challenge diet.

In a paired 2×2 crossover experimental design, Zebeli et al. (2012) dosed 8 primiparous Holstein cows every day for 2 periods of 21 d with either saline (control) or 35 mL (10^8 cfu/mL) suspension of *M. elsdenii*. A decline in rumen pH in the hours post-feeding did occur for both control and *M. elsdenii* dosed cows, but pH remained ≥ 5.8 for both. In addition, there was no treatment difference in the lactic acid concentration in *M. elsdenii*-dosed animals compared to control animals. Total rumen VFA between *M. elsdenii*-dosed animals and control did not differ, but acetate, isobutyrate, and isovalerate were lower and valerate and butyrate were higher for *M. elsdenii*-dosed cows. Milk yield did not differ between cows inoculated with *M. elsdenii* and control cows. There was a tendency for lactose percentage to be lower ($P = 0.06$) and the somatic cell count (SCC) (cell/ml) to be higher ($P = 0.06$) in *M. elsdenii*-dosed cows compared to control cows. Blood was sampled on d 1, 3, 5, 7, and 10 of each period prior to the morning feeding and also sampled every 2 h after the morning feeding on d 10. Of the metabolites measured, only the plasma nonesterified fatty acids (NEFA; lower) and cholesterol (higher) concentrations differed with *M. elsdenii* treatment. There was no treatment effect on BHBA, and BHBA concentrations increased in both groups postprandial.

Henning et al. (2011) evaluated the effects of dosing *M. elsdenii* to early lactation cows fed either a 60 or 70% concentrate diet. Half of the cows were dosed with 250 ml of

a 10^{11} cfu suspension of *M. elsdenii* on the day of calving and on d 10 and 20 postpartum and the other half were control cows (not dosed). Trial measurements were recorded until 80 DIM. Dry matter intake did not differ between *M. elsdenii*-dosed and control cows, but *M. elsdenii*-dosed cows tended to produce more milk. Cows dosed with *M. elsdenii* also had higher BW and BCS than control cows, and these differences were greater for cows on the higher concentrate diet (70%). No difference in milk protein occurred between control and dosed cows. For the cows receiving the 60% concentrate diet, the *M. elsdenii*-dosed cows had significantly higher milk fat percentage than the control cows; however, milk fat percentage did not differ between control and dosed cows on the 70% concentrate diet or overall between the 2 dosing treatments.

F. Summary

Although *M. elsdenii* can be introduced into the rumen and establish populations capable of preventing lactate accumulation and acidosis, there is variation in animal response. Small animal numbers or a diet not sufficient enough to induce acidosis conditions may explain why some studies did not observe a difference in lactate accumulation or rumen pH between *M. elsdenii*-inoculated and control animals. The large individual variability in an animal's susceptibility to rumen acidosis may have been an important factor in the studies with a lower number of animals (Mohammed et al., 2012). Despite the inconsistent results in lactic acid concentration and rumen pH between *M. elsdenii* treated and control animals, shifts in the rumen VFA profile have occurred. Animals administered with *M. elsdenii* tended to have higher butyrate and lower acetate and propionate concentrations, which suggests the rumen pH is low enough to favor the

iD-LDH pathway over the acrylate pathway. The effect of *M. elsdenii* on animal performance is unclear. Milk production and composition results are inconsistent and more research is needed. In addition, few animal trials have evaluated the effect of *M. elsdenii* administration on blood metabolites and cow health.

As evident from this discussion, rumen health has a significant impact on feed intake and subsequent animal performance. The effects of *M. elsdenii* administration have been observed in feedlot steers, wethers, and post-partum dairy cows; however, there are no published studies in which *M. elsdenii* was administered to pre-fresh cows or administered to cows pre-fresh and at calving. Previous work with dairy cows has shown little promise for *M. elsdenii* to increase milk yield, and Hagg et al. (2010) observed a decline in milk fat percentage in dosed cows. More investigation is needed to understand why there was a decline in milk fat percentage and no effect on milk yield. The trend for increased milk production efficiency observed by Aikman et al. (2011) in *M. elsdenii*-dosed cows in contrast to the lack of difference between dosed and control cows observed by Hagg et al. (2010) and Zebeli et al. (2012) suggests that the effect of *M. elsdenii* differs by diet composition. Lower concentrations of NEFA and higher molar proportions of propionate in *M. elsdenii*-dosed cows versus control cows may indicate less lipolysis and a decreased risk of ketosis in fresh cows with *M. elsdenii* inoculation, but more research is needed since there was no treatment difference in plasma BHB concentrations (Zebeli et al., 2012).

G. Hypotheses

Based on the results of in vitro studies with *Megasphaera elsdenii*, there is promise for improved performance in dairy cows during high-stress transition periods; however, none of the present in vivo studies have shown a significant difference in performance between treatments. We hypothesized that administering *M. elsdenii* would improve milk yield and decrease the incidence of metabolic disease in early lactation dairy cows. In addition, we also hypothesized that administering *M. elsdenii* both prepartum and at calving, compared to previous studies in which *M. elsdenii* was only administered postpartum, would improve performance and health further than either a single-dose prepartum or postpartum.

CHAPTER 3: MATERIALS AND METHODS

Experimental Design, Treatments, and Diets

Primi- and multiparous Holstein cows (n=162) at the Miedema Dairy Farm (Circleville, OH) were enrolled in the trial beginning August 22, 2012. Dry cows and heifers were placed on the trial at ~14 d prepartum until the last cow calved on November 11, 2012. The trial ended on February 8, 2013 when the last cow reached 90 DIM. Cows were followed until 90 DIM for milk production and health data and until 150 DIM for reproduction data. The study was conducted as a randomized complete block design. Cows and heifers were blocked based on expected calving date and parity. The researchers chose to use a commercial farm rather than a university farm in order to have higher animal numbers and potential for increased metabolic disease prevalence.

Within each block cows were randomly assigned to 1 of 4 treatments: 1) control (no dose) (n=44), 2) 200 mL Lactipro (*M. elsdenii*, 1×10^8 cfu/mL; MSBiotec, Littleton, CO) when cows entered (~ -14 DIM) the close-up pen (n=45), 3) 200 mL Lactipro at 1-3 d postpartum (n=39), and 4) 200 mL Lactipro prepartum and another 200 mL at 1-3 d postpartum (n=34). The 200 mL doses of Lactipro were administered orally while cows were in headlocks during the morning feeding. The researchers chose not to have a placebo dose in order to more closely mimic actual on-farm use of the Lactipro product. The different number of animals per treatment was due to early removal of some animals

by the farm manager and failure of animals to be dosed postpartum. Cows were housed in 1 of 2 dry cow groups prepartum in a 4-row freestall barn and were moved, within the same facility, into a fresh cow group postpartum. All cows had *ad libitum* access to a TMR, which was mixed and delivered every day at 0900 h. Close-up cows were fed a diet formulated with 72% forage (63:37 corn silage and wheat straw), and upon calving, cows entered the high group with a diet formulated for 40% forage (90:10 corn silage and wheat straw). Ingredient compositions for both the dry and high cow TMR are listed in **Table 1**. All cows had *ad libitum* access to water.

BCS and Weight

Cows were body condition scored (1 to 5 on a quarter unit scale; 1 = thin, 5 = fat) prepartum (~ -14 DIM) and at 30, 60, and 90 DIM. The principal investigator did the majority of the body condition scoring with the assistance of 2 other research personnel that were trained by the principal investigator. The BW was estimated concurrently with the second blood collection day (7 to 14 DIM) via weigh tape (Coburn Co., Whitewater, WI).

Urine and Blood Metabolites

In order to determine the metabolic status of the cows pre- and postpartum, blood samples were collected from the median coccygeal vein or artery 3 to 5 d prior to the expected calving date and again at 7 to 14 DIM for analysis of BHBA and NEFA. The blood samples were collected in 10 mL Vacutainer serum collection tubes (BD

Vacutainer, Franklin Lakes, NJ). Blood tubes were immediately placed on ice until they were centrifuged at 2000 x *g* at 4 °C for 20 min. The serum was then decanted and divided between 2 plastic disposable culture tubes. Serum samples were frozen at -20 °C until analysis. The NEFA concentrations were determined using an enzymatic colorimetric method assay (Wako Diagnostics, Richmond, VA). The BHBA concentrations were analyzed using an enzymatic assay kit (Sigma-Aldrich Co. LLC, St Louis, MO). All samples were analyzed in duplicate for both NEFA and BHBA assays. The concentration of BHBA and NEFA were calculated using the absorbance values obtained from the microplate spectrophotometer (Molecular Devices Corp., Sunnyvale, CA). Urine ketones (acetoacetate) were measured at 7 to 14 DIM using Ketostix (Bayer Corporation, Leverkusen, Germany) for comparison with blood BHBA and milk fat percentage.

Health and Reproduction Events

Reproduction and health events were monitored until 150 and 90 DIM, respectively. Dairy personnel recorded all reproduction and health events in DairyCOMP 305 (Valley Agricultural Software, Tulare, CA).

Feed Sampling and Analyses

Each week, a sample of the dry cow TMR, the high cow TMR, and individual dietary components were collected and frozen at -20°C for later nutrient analysis. The weekly TMR samples for each diet were dried at 55°C for 48 h and ground through a 1-

mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). Weekly TMR samples were analyzed for DM (AOAC, 1990), crude protein (N x 6.25) (AOAC, 1990; Kjeldahl N, Foss 220 Kjeltac Auto Distillation, Eden Prairie, MN), and ash (AOAC, 1990). The NDF content of the TMR samples was analyzed using an ANKOM 200 fiber analyzer (Ankom Technology, Macedon, NY) based on procedures of Van Soest et al. (1991) using heat-stable α -amylase and sodium sulfite. The weekly TMR samples were composited by month for mineral and fat analyses. Mineral concentrations of the composited TMR samples were analyzed using inductively coupled plasma emission spectrometry by The Ohio State University STAR laboratory (Wooster, OH). Composite TMR samples were analyzed for fat content by ether extraction (AOAC, 2006) at Cumberland Valley Analytical Services (Maugansville, MD).

Milk Collection and Analyses

Cows were milked 3 times daily (0500, 1300, and 2100 h) and daily milk yield was recorded until 90 DIM using Afimilk (Kibbutz Afikim, Israel) and DairyCOMP 305 (Valley Agricultural Software, Tulare, CA) data collection. Daily milk yield was averaged weekly for each cow for milk production analysis. Peak milk and week of peak milk were calculated from the weekly milk yield averages. Milk composition data were collected from the monthly DHI (DHI Cooperative, Inc., Columbus, OH) samples taken from the first daily milking during the first two test dates postpartum. The DHI composition analyses of milk fat and protein were determined by infrared spectroscopy (B2000 Infrared Analyzer, Bentley Instruments, Chaska, MN), and SCC were analyzed using a Bentley Somacount 300 (Bentley Instruments, Chaska, MN).

Statistical Analysis

Data were analyzed using PROC MIXED of SAS (SAS Institute, 2004) as a randomized complete block design with treatment, time, and parity as fixed effects and block as the random effect. Milk yield, milk fat and protein percentages, SCC, projected 305 d milk production, and BCS were analyzed as repeated measures. An autoregressive order 1 covariate matrix was used. Prepartum BCS was used for covariate adjustment of BCS data. The PDIFF option in the LSMEANS statement was used to separate means. Significant differences were declared at $P \leq 0.05$ and trends at $P \leq 0.10$. Nonsignificant 2- and 3-way interactions were pooled into the error term.

CHAPTER 4: RESULTS

Experimental Design, Animals, and Treatments

Cows in the prepartum only and pre- and postpartum treatments were dosed, on average, 8 d prior to calving, which varied slightly from the intended dosing time of 14 d prepartum. Cows were not included in data analysis if they were culled from the herd or died before the 7 to 14 DIM measurements could be collected. In addition, entire blocks were deleted if they contained fewer than 2 cows. The postpartum only and pre- and postpartum treatments contained the fewest number of cows, 39 and 34 cows respectively, while the control and prepartum only treatment contained 44 and 45 cows respectively.

Feed Composition

Chemical composition of the 2 diets is shown in **Table 2**. Differences between the formulated and actual nutrient content of the diets occurred. Variability between the calculated and analyzed CP and NDF concentrations for the dry cow TMR did exist. On a DM basis, the analyzed CP for the dry cow diet was 14% lower than the calculated and the analyzed NDF value was 9% higher than the calculated value. Differences between the calculated and analyzed dry cow TMR were observed for K, Ca, Mg, S, Cu, and Mn, of which Cu was the only one in which the analyzed amount was greater. The minerals with the most variability in the high cow TMR were K, P, Mg, S, Cu, Mn, and

Zn, of which K and Mn were the only 2 in which the analyzed amount was less than the calculated. Overall, dietary concentrations of nutrients in the actual diets met or exceeded NRC (2001) recommendations.

BCS and Weight

The BCS did not differ by treatment (**Table 3**), but it differed by DIM (**Figure 1**). No difference in BCS occurred between 30 and 60 DIM, but there was a difference for both 30 and 60 DIM to be lower compared to 90 DIM. The higher BCS at 90 DIM suggests the cows were regaining the body condition they had lost postpartum. There was no treatment by day interaction for BCS ($P > 0.10$). Postpartum BW measured between 7 and 14 DIM did differ by treatment. Cows dosed pre- and postpartum had higher ($P < 0.05$) BW than either of the prepartum or postpartum dosing treatments. The control cows also had a higher ($P < 0.05$) BW than the prepartum dosed cows.

Milk Production and Composition

Milk production and composition data are provided in **Table 3**. Daily milk yield did not differ by treatment, with cows producing an average of 39.7 kg/d. As expected, a week difference by DIM was observed for daily milk yield ($P < 0.01$) (**Figure 2**); however, there was no treatment by week interaction for daily milk yield ($P > 0.10$). Projected 305 d milk yield did not differ by treatment and test-day, and no treatment by test-day interaction was observed. Peak milk yield did not differ by treatment, and cows across all treatments peaked at an average of 45.6 kg/d. Treatment by parity interactions

($P < 0.05$) occurred for daily milk yield (**Figure 3**) and for peak milk (**Figure 4**) and differences among treatments were most pronounced for cows with ≥ 3 lactations. The mature cows dosed prepartum had higher daily milk yield and peak milk than the control and pre- and postpartum dosed cows but did not differ from the postpartum dosed cows. Week of peak milk did not differ by treatment and occurred between 8 and 9 wk in lactation.

Milk fat percentage did not differ by treatment, and milk fat percentage averaged 3.63% across all treatments. As expected, the first test-day milk fat percentage was higher ($P < 0.01$) than the second test-day, 3.88% and 3.38%, respectively. The average milk protein percentage across all treatments was 3.08% and did not differ by treatment. Milk protein percentage was higher ($P < 0.01$) on the first test-day than the second test-day (3.16% and 2.99%, respectively). There was no interaction of treatment by time for milk fat or protein percentages ($P > 0.10$), but a treatment by parity interaction ($P < 0.01$) occurred for milk protein (**Figure 5**). Milk protein percentage was higher for 2nd lactation cows dosed prepartum than control or cows dosed pre- and postpartum but did not differ from cows dosed postpartum. Control and pre- and postpartum dosed cows with ≥ 3 lactations had higher milk protein percentages than those dosed only prepartum but were not different from cows dosed only postpartum. The SCC did not differ by treatment or DHI test-day and averaged 333×10^3 cells/ml across all treatments. A treatment by DHI test-day interaction occurred ($P = 0.05$), but this was mainly the result of 3 postpartum dosed cows having SCC between 2,000,000 and 5,000,000 cells/ml.

Urine and Blood Metabolites

The results for urine and blood metabolites are listed in **Table 3**. Urine ketones (acetoacetate) did not differ among treatments. Prepartum blood was collected, on average, 6 d prior to calving which varied slightly from the intended sampling time of 3 to 5 d prepartum. Neither the prepartum nor postpartum NEFA concentrations differed for cows dosed with *M. elsdenii* or control cows. The prepartum and postpartum BHBA concentrations also did not differ among treatments.

Reproduction and Health

All reproduction and health events are shown in **Table 4**. The incidence of monitored health events did not differ among treatments, and as expected, the 2 most prevalent health events were mastitis and lameness. A new episode of mastitis was defined as an episode occurring 14 d after the treatment of a previous episode as described by Adkinson et al. (1993). The farm personnel did not record any incidences of ketosis, SARA, or displaced abomasum for cows during the trial.

The DIM at 1st service did not differ by treatment, and on average, cows were first serviced at 74 DIM across all treatments. There was no difference in the number of cows pregnant by 150 DIM and approximately 68% of the cows in each treatment were confirmed pregnant by this stage of lactation. Of the confirmed pregnant cows by 150 DIM, it took an average of 1.65 services per conception, and there was no difference among treatments for the number of services per conception.

CHAPTER 5: DISCUSSION

BCS and Weight

The suggested BCS score at calving for optimal milk production is 3.5, but little difference in milk production exists between a BCS of 3.0 and 3.5 (Roche et al., 2009). In addition, cows should not lose more than 0.5 to 1.0 units postpartum (Roche et al., 2009). The cows on our study had a BCS of 3.24 in the 2 wk prior to calving and only lost 0.3 units between the 2 wk prepartum and 30 DIM. Thus, the BCS of cows in our study conformed to industry recommendations for maximum milk production. Similar to our study, Hagg et al. (2010) did not observe a difference in BCS between *M. elsdenii*-dosed and control cows. Conversely, Henning et al. (2011) reported that cows dosed with *M. elsdenii* tended to have higher BCS than control cows. The treatment difference in BW observed in our study is not likely due to a true treatment difference, as cows were not blocked by BW, a covariate for BW was not included in the analysis, and the measurement of BW was by heart girth tape. The effect of dosing *M. elsdenii* on BW is inconsistent in the existing literature. Aikman et al. (2009) reported no treatment difference in BW for dosed or control cows, but cows dosed with *M. elsdenii* tended to either lose less or gain more weight than control cows. In contrast, in a study by Henning et al. (2011), cows dosed with *M. elsdenii* had higher BW than control cows. In addition, the difference in BW and BCS was accentuated in high-producing cows on a higher

energy diet (70% concentrate). It may be beneficial for future studies to evaluate changes in BW, in addition to BCS, when assessing the effect of dosing *M. elsdenii* to cows in early lactation.

Milk Production and Composition

Administration of *M. elsdenii* had little effect on milk yield or milk composition in this study. Similarly, other studies (Aikman et al., 2009; Hagg et al., 2010; Aikman et al., 2011) also have revealed no difference in milk yield when cows were dosed immediately postpartum. Henning et al. (2011) observed a trend for higher milk yield by cows dosed with *M. elsdenii* on 10 and 20 DIM compared to control cows. In our study, there was a treatment by parity interaction for milk yield and peak milk in which mature cows (≥ 3 lactations) dosed prepartum produced more milk and peaked higher than control cows and cows dosed with *M. elsdenii* both pre- and postpartum. The lack of a response in milk production may be contributed to the composition of the lactating cow diet and good herd management. The 59% concentrate in the lactating cow diet in our study is comparable to the low concentrate diets (60% concentrate) fed by Hagg et al. (2010) and Henning et al. (2011). Because neither of these studies observed a difference in control and *M. elsdenii*-dosed cows for milk yield suggests that cows on a lower concentrate (low NSC) diet did not benefit as much from *M. elsdenii* administration compared to those fed the higher NSC diet. In addition, proper feeding management, such as uniform feed mixing, no empty bunks, and optimal fiber length also may minimize potential differences between control and *M. elsdenii* dosed cows.

The milk fat percentage did not differ between cows dosed with *M. elsdenii* and control cows, which is in agreement with the studies by Aikman et al. (2009) and Zebeli et al. (2012). Conversely, Aikman et al. (2011) observed a trend for milk fat concentration to be reduced in cows dosed with *M. elsdenii*. Although Hagg et al. (2010) did not observe an overall treatment difference between control cows and cows dosed with *M. elsdenii*, there was a significant difference in milk fat percentage between dosed and control cows fed a high concentrate diet (70% concentrate). The milk fat percentages in our study are consistent with the national average of 3.7% for Holstein cows and do not indicate there was a high risk for SARA. However, it must be noted that cows in our study were in early lactation and higher milk fat percentages are expected due to the mobilization of adipose tissue. In addition, the lack of a milk fat depression with the administration of *M. elsdenii* suggests that this strain may not be associated with the production of *trans*-10,*cis*-12 conjugated linoleic acid, unlike some of the *M. elsdenii* strains studied by others (Kim et al., 2002; Palmonari et al., 2010; Weimer et al., 2010). The suggestion that *M. elsdenii* produces *trans*-10,*cis*-12 conjugated linoleic acid is refuted by Maia et al. (2007) and Wallace et al. (2007). Neither of the 2 *M. elsdenii* strains in the Maia et al. (2002) study were found to produce *trans*-10,*cis*-12 conjugated linoleic acid. Of the *M. elsdenii* strains studied by Wallace et al. (2007), including the strain used by Kim et al. (2002), none of the *M. elsdenii* produced *trans*-10,*cis*-12 conjugated linoleic acid.

Similar to our observation of no difference in milk protein percentage between control and *M. elsdenii*-dosed cows, none of the other dairy cattle studies that evaluated milk protein observed a difference between control and dosed cows. The treatment by

parity interaction observed in our trial is likely due to a dilution effect, as similar trends were observed for milk yield and peak milk. In contrary to our study, where there was no observed difference in SCC for control or dosed cows, Zebeli et al. (2012) reported a trend for *M. elsdenii* dosed cows to have higher SCC than control cows. The authors did note that both the control and dosed cattle had SCC below 150,000 cells/ml. In agreement with Hagg et al. (2010), there was no difference in the 305-d predicted milk between cows dosed with *M. elsdenii* and control cows. Overall, the administration of *M. elsdenii*, regardless of dosing day relative to calving, had little effect on milk yield and composition in this study. However, the treatment by parity interaction may suggest higher producing cows with higher DMI may benefit from dosing with *M. elsdenii*.

Urine and Blood Metabolites

Urine ketones and plasma NEFA and BHBA are commonly used as indicators of energy status in early lactation dairy cattle. The concentration of urine ketones in our study suggests the incidence of ketosis in our study was low across all treatments. Carrier et al. (2004) suggested 15 mg/dl as the cutoff point for subclinical ketosis when using Ketostix to measure urine ketones. Based on this cutoff point, only 2.5% of all cows in the study experienced clinical ketosis, and cows with ≥ 15 mg/dL were 8.33, 14.3, 14.3, and 0% for treatments, respectively. The prepartum and postpartum NEFA and BHBA concentrations also suggest that cows on our trial did not suffer from severe NEB. The NEFA concentrations in this study did not exceed the thresholds of ≥ 0.3 mEq/L and ≥ 0.6 mEq/L for prepartum and postpartum, respectively (Ospina et al., 2010). The BHBA

concentrations in our study did not exceed the threshold of 1,200 to 1,400 $\mu\text{mol/L}$ (12.4 to 14.4 mg/dl) for postpartum cows (Duffield et al., 2009; Suthar et al., 2013).

Although not significant, the prepartum and postpartum dosing treatments had the highest prepartum and postpartum NEFA, which corresponds to the higher milk production for these 2 treatments for the mature cows because higher producing cows have a higher energy demand. Zebeli et al. (2012) measured preprandial NEFA and BHBA and found no difference between *M. elsdenii*-dosed cows and control cows. However, there was a significant postprandial effect in which control cows had higher plasma NEFA than dosed cows. The low urine ketones and NEFA and BHBA concentrations in our study are in agreement with the absence of any reported cases of ketosis. In addition, an elevated milk fat percentage of one standard deviation above the average, about 1 percentage unit, is suggestive of ketosis in early lactation dairy cows (Eastridge, 2012). Based on this, the cows in our study had low prevalence of ketosis; the average milk fat percentage for all cows in the first 30 DIM was only 4.15%, and the average for the first 90 DIM was 3.63%. The effect of dosing with *M. elsdenii* on urine ketones and plasma NEFA and BHBA remains unclear but may indicate improved energy status.

Reproduction and Health

Evidence that dosing cows with *M. elsdenii* NCIMB 41125 reduces the incidence of health events is inconsistent. In the study by Leeuw et al. (2009), dosing *M. elsdenii* NCIMB 41125 to feedlot steers on low roughage diets reduced the incidences of bloat and diarrhea. The number of animals treated for illnesses also was numerically less for

the steers dosed than control (15 and 26, respectively). Of the cows culled for a decline in milk production, DMI, or illness in the lactating cow study by Hagg et al. (2010), fewer cows were culled from the *M. elsdenii* treatments than the control treatment. Four and 7 cows from the low and high control treatments, respectively, and 1 from each of the low and high dosed treatments were culled. In our study, dosing early lactation cows with *M. elsdenii* did not appear to reduce the occurrence of health problems. The herd personnel reported no incidences of SARA, ketosis, or displaced abomasum. The absence of the incidence of SARA in this study may suggest that the lactating diet did not have sufficient NFC to cause a rumen challenge and thus mitigated any possible effect of dosing with *M. elsdenii*.

Reproduction success in dairy cattle can be linked to nutrition and health status; thus, the NEB that cows experience in the first few weeks of lactation could adversely affect reproductive performance. Domecq et al. (1997) reported that cows were 1.17 and 1.36 times less likely to conceive if cows lost 0.4 or 0.8 BCS units, respectively, in the first months after calving compared to those cows that did not change in BCS. The services per conception in our study were positively below the recommended benchmark of 2.5. The low number of services per conception in our study indicates that cows may not have experienced a NEB severe enough to adversely affect reproduction. The suggested goal for DIM at 1st service is 50 to 70 d, and the cows in our trial were slightly past this goal (average of 74 DIM). The DIM at 1st service observed in our study is likely due to farm-specific reproduction protocols because there were low incidences of health events.

Implications

As food-animal production industries move away from antibiotic use and towards other animal health practices, such as DFM, it is important to understand the effects that DFM have on milk production, milk composition, and animal health. From this study and others investigating the effect of *M. elsdenii* administration in dairy cattle fed typical transition diets, *M. elsdenii* does not appear to increase the milk yield of early lactation cows or reduces health events. However, dosing with *M. elsdenii* either 2-wk prepartum or 1 to 3 d postpartum increased the milk production of the higher producing mature cows (≥ 3 lactations). The administration of *M. elsdenii* both prepartum and immediately following calving did not further improve cow performance and suggests that only one dose of *M. elsdenii* prepartum is potentially valuable. Future studies should evaluate the effect of dosing both prepartum and postpartum with cows fed a diet with higher concentrations of starch or NFC either prepartum or postpartum.

Conclusions

The milk yield and composition were similar among treatments. Dosing with *M. elsdenii* prepartum may improve ruminal conditions of high-producing (higher parity) transition cows whereby milk yield is increased. Although administration of *M. elsdenii* did not appear to affect overall cow performance, concentrations of dietary starch and NDF fed both prepartum and postpartum may affect animal response to dosing with *M. elsdenii*.

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APPENDIX A: TABLES

Table 1. Ingredient composition of dry cow and lactating (high) cow diets (% of DM)

Item	Dry	High
Wheat straw	26.6	4.01
Corn silage	45.7	36.0
Corn grain, ground	NA ²	13.4
Soybean meal and whey blend	NA	8.76
Soybean meal, 48% CP	11.2	4.46
Soybean hulls	NA	10.1
Amino Plus ¹	NA	5.54
Wet distillers grains, 35% DM	NA	5.53
Wet brewers grains	12.4	6.46
Dry cow grain mix	4.10	NA
High cow grain mix	NA	5.78

¹AMINOPLUS[®], Ag Processing Inc. [©], Omaha, NE

²NA = Not applicable

Table 2. Chemical composition of the diet formulated by the herd nutritionist and the chemical composition of the actual diet fed

Variable	Formulated		Actual	
	Dry Cows	High Cows	Dry Cows	High Cows
DM, %	45.6	48.5	42.4	47.5
CP, % of DM	15.5	18.9	13.3	17.6
NDF, % of DM	44.9	31.5	49.5	31.2
NFC ¹ , % of DM	28.1	40.0	26.6	40.8
Starch, % of DM	16.2	26.2	NA ²	NA
Fat, % of DM	3.1	3.83	2.91	3.59
Ash, % of DM	NA	NA	7.73	6.80
K, %	1.22	1.51	1.06	1.20
P, %	0.32	0.34	0.32	0.40
Ca, %	1.27	0.75	1.01	0.79
Mg, %	0.34	0.28	0.31	0.34
S, %	0.26	0.22	0.22	0.24
Cu, ppm	29.9	20.1	33.1	21.8
Fe, ppm	NA	NA	309	295
Mn, ppm	141	85.1	113	74.2
Mo, ppm	NA	NA	2.90	2.33
Zn, ppm	122	80.2	123	93.4

¹NFC = non-fiber carbohydrate = 100-(%NDF + %CP + %fat + %ash)

²NA = Not available

Table 3. Least square means of body condition score, body weight, urine ketones, plasma NEFA and BHBA, milk production, and milk composition in cows not dosed and cows dosed with *M. elsdenii* ~14 d prepartum, 1 to 3 d postpartum, or ~14 d prepartum and 1 to 3 d postpartum

Item	Treatment				SEM	P-value		
	Control	Prepartum Dose	Postpartum Dose	Pre & Post Dose		TRT	Time [†]	TRT x Time
BCS ¹	3.01	3.05	2.99	2.92	0.05	0.28	0.01	0.83
BW ¹ , kg	685 ^a	656 ^b	657 ^{ab}	690 ^{ac}	13.6	0.04	NA ¹	NA
Milk, kg/d	39.7	39.7	39.5	39.8	0.97	1.00	0.01	0.70
Projected 305d Milk, kg	10,371	10,405	10,317	10,362	216	0.99	0.28	0.49
Peak milk, kg/d	45.2	45.1	45.5	46.1	1.1	0.93	NA	NA
Peak milk, wk	9.1	8.8	8.8	8.6	0.4	0.87	NA	NA
Fat, %	3.55	3.62	3.75	3.55	0.11	0.51	0.01	0.75
Protein, %	3.07	3.04	3.09	3.07	0.04	0.86	0.01	0.68
SCC ¹ , 10 ³ cells/ ml	277	349	381	326	96	0.88	0.31	0.05
Urine ketone, mEq/L	2.78	7.43	6.43	1.30	2.39	0.23	NA	NA
Prepartum NEFA ¹ , mEq/L	0.28	0.30	0.26	0.25	0.03	0.74	NA	NA
Postpartum NEFA, mEq/L	0.35	0.45	0.40	0.35	0.04	0.25	NA	NA
Prepartum BHBA ¹ , mg/dl	11.6	10.8	9.76	10.2	0.80	0.38	NA	NA
Postpartum BHBA, mg/dl	11.5	12.0	11.2	12.2	0.45	0.43	NA	NA

^{abc}Means in the same row with different superscripts differ ($P < 0.05$).

¹BCS= Body condition score, BW= body weight, SCC= somatic cell count, NEFA= nonesterified fatty acids, BHBA= β -hydroxybutyrate, and NA = not applicable

[†]Time relates to the 0, 30, 60, and 90 DIM for BCS; week for milk yield; and DHI test day for 305 d predicted milk, milk fat, milk protein, and SCC

Table 4. Incidence of health events within each treatment (% of cows with health event) until 90 DIM and least square means for reproductive performance until 150 DIM for cows not dosed and cows dosed with *M. elsdenii* ~14 d prepartum, 1 to 3 d postpartum, or ~14 d prepartum and 1 to 3 d postpartum

Item	Treatment				SEM	P-value
	Control	Prepartum Dose	Postpartum Dose	Pre & Post Dose		TRT
<i>Health Events</i>						
RP ¹	2.27	2.22	2.56	2.94	2.48	1.00
Milk fever	2.27	2.22	2.56	0.0	2.15	0.85
Lame	2.27	6.67	0.0	8.82	3.21	0.22
Hoof abscess	0.0	0.0	2.57	5.89	2.12	0.19
Diarrhea	0.0	2.22	0.0	0.0	1.24	0.46
Mastitis	22.8	13.4	8.05	18.0	8.39	0.62
<i>Reproduction</i>						
DIM at 1 st Service	74.0	74.2	74.6	71.5	1.55	0.55
Pregnant by 150 DIM, % cows	68.2	66.7	76.9	61.8	7.38	0.57
Services/conception [†]	1.53	1.57	1.77	1.71	0.15	0.61

¹RP= retained placenta and HHW= hairy heel wart

[†]Services/conception= cows confirmed pregnant by 150 DIM

APPENDIX B: FIGURES

^{ab}Days in milk with different superscripts differ ($P < 0.05$).

Figure 1. Body condition score from 2 wk prepartum to 90 DIM for control, prepartum dosed, postpartum dosed, and pre- and postpartum dosed cows.

Figure 2. Milk production from wk 1 to 13 of lactation for control, prepartum dosed, postpartum dosed, and pre- and postpartum dosed cows.

^{ab}Treatments with different superscripts differ ($P < 0.05$).

Figure 3. Treatment by parity interaction ($P < 0.05$) for milk yield for control, prepartum dosed, postpartum dosed, and pre- and postpartum dosed cows.

^{ab}Treatments with different superscripts differ ($P < 0.05$).

Figure 4. Treatment by parity interaction ($P < 0.05$) for peak milk for control, prepartum dosed, postpartum dosed, and pre- and postpartum dosed cows.

^{ab}Treatments with different superscripts differ ($P < 0.05$).

Figure 5. Treatment by parity interaction ($P < 0.05$) for milk protein percentage for control, prepartum dosed, postpartum dosed, and pre- and postpartum dosed cows.