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Optimizing the adaptation rate of feedlot steers dosed with *Megasphaera elsdenii* NCIMB 41125 and fed high starch diets.

By

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Submitted in partial fulfillment of the requirements for the degree

Magister Scientiae Agriculturae – MSc (Agric)
Animal Science: Animal Nutrition

In the faculty of natural and Agricultural Sciences
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Pretoria

March, 2010

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Declaration

I hereby declare that this dissertation, submitted for the MSc (Agric) Animal Nutrition degree at the University of Pretoria, is my own work, conducted under the supervision of Prof L.J. Erasmus and that it has not previously been submitted by me for a degree at any other University.

A.A. Campbell

Pretoria

March 2010

Acknowledgements

Throughout the duration of this trial as well as afterwards I was lucky enough to have the assistance and support of so many people along the way. Truth be told it is extremely difficult to thank each an every one in a few short lines and do the necessary justice to the contribution which each and every one made to make this a successful and enjoyable process.

To single out an individual who made the difference in this experience seems unfair, but truthfully I have much to thank to one such individual. Therefore I would like to personally thank Frans Hagg of KK Animal Nutrition, for all his hard work and long hours and his exemplary commitment to the successful on going of this trial. Throughout, he put his experience and assistance into the trial making the entire experience a far easier one for all as well as adding the humor and knowledge that made this trial the enjoyable and fruitful one it ended up to be.

Also to Prof. L.J. Erasmus, my supervisor of the trial from the University of Pretoria, I would like to thank him for his support and guidance throughout. Never short of an idea, Prof. Erasmus was always there to lend a hand or an ear should an unforeseen circumstance arise in such a trial as so often happens.

Then to Dr. Pieter Henning for always being there and for his wealth of knowledge and a key eye for improvement. He was always a ready sounding board for Frans and myself and was always ready to step in as the need arose.

Then to the most important part of this trial, Dr. Klaasjan Leeu and the staff at the Agricultural research centre (ARC), Irene. This group of individuals could so easily have been overlooked for acknowledgement but without them none of this would ever have

been possible. Always eager to help and never allowing for a dull moment, they all were a pleasure to work with. Even during the long hours of mixing feed weekly or during feeding of the steers each and every morning all at hand were in high spirits and eager to assist to what in the end was a smooth project.

Then to the Nutrilab team at the University of Pretoria, with special thanks to Elise Ferreira and Tilla Basson. Thank you both for all the fond memories, endless support and guidance. Without you and all the encouragement, I would never have had the courage to get my analysis done as accurately or quickly and painlessly as I did in the end. The lab work carried out behind the scenes of a trial can so easily go without the necessary acknowledgement but thanks to your enthusiasm and willingness, this will never go forgotten by me.

Lastly but definitely most importantly I would like to thank all my friends and family for the belief that without it, I would never have been able to accomplish what I did. The endless support and good wishes from you all gave me the encouragement to get through the long days during the trial and the even longer nights afterwards. You all were always there to pick me up when I was down on for that a special thanks to Anneke who throughout never let me drop my head. Also for the patience when I was in one of my moods, to understand what I was going through and throughout, stand by me, I thank you all for that.

Last but definitely not least, to the divine power for giving me firstly the ability to complete not only this trial but also my studies as well as the blessings along the way which without, none of this would ever have been possible.

Summary

Optimizing the adaptation rate of feedlot steers dosed with *Megasphaera elsdenii* NCIMB 41125 and fed high starch diets.

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Rumen acidosis is a common problem under feedlot conditions where cattle change from a roughage-based to a high concentrate-based diet. It is associated with an imbalance between lactic acid producing and lactic acid utilizing bacteria. *Megasphaera eldenii* is an important lactic acid utilizing bacteria which can now be produced for commercial use and supplemented for the prevention of lactic acid build up in the rumen and subsequent acidosis. The adaptation period is required in order for numbers of lactate utilizers such as *M. elsdenii* to increase to levels effective against subsequent build up of lactic acid. The purpose of this trial is to identify the effects of a strain, NCIMB 41125 (MeCH4), of *Megasphaera elsdenii* on the adaptation period of feedlot cattle on diets formulated for high starch levels. MeCH4 was isolated from the rumen of cattle adapted to high starch diets. By converting lactic acid in the rumen to volatile fatty acids (VFA's) such as

butyrate, *M. elsdenii* should be able to at least reduce the adaptation phase, if not totally eliminate the need for it without effects on intake patterns, feed conversion rates, health and carcass yield. . If results are positive, this strain of *M. eldenii* will have great economic implications to all feedlot farmers. There may be one major conclusion to be drawn from this trail and this was the fact that a decrease in adaptation days to reach the high concentrate grower diet had no statistically significant influence on key performance parameters relevant to the feedlot industry However, possibly the most attractive implication of these results is the lower need for roughage and therefore the implications tied in with the buying in and storage thereof.

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List of Abbreviations

Abbreviation	
ADF	Acid detergent fiber
ADG	Average daily gain
ARC	Agricultural research council
BW	Body weight
Chop	Hominy chop
CP	Crude protein
DFM	Direct fed microbial
DM	Dry matter
DMI	Dry matter intake
DRTD	Days to reach top diet
EE	Ether extract
FCR	Feed conversion ratio
IBR	Infectious bovine rhinotraceheitis
ME	Metabolizable energy
MRT	Mean retention time
NDF	Neutral detergent fiber
NSC	Non structural carbohydrates
NWG	Net weight gain
pHi	Internal pH
RFC	Rapidly fermentable carbohydrates
SOP	Standard operating procedures
TMR	Total mixed ration
VFA	Volatile fatty acids

Chapter 1: General Introduction

Ruminant livestock production proves to be far more economical when cattle are fed grain-based diets as well as other energy dense feeds as opposed to forage even with ever increasing prices of grains and other feedlot diet components (Leedle, 2006). However ruminant's digestive tracts were not designed to process these high energy feeds which can easily cause digestive upsets. Although the rumen microbe population is able to adapt if given enough time when small, continual changes are made to the diet, the economic viability will be severely compromised by the resultant losses in production as almost no producers can afford the time length of this natural adaptation phase (Leedle, 2006). Feeding regimes are therefore required to be changed as quickly as possible without subsequent microbial upsets which may manifest themselves in the form of acute or sub-acute rumen acidosis (Leedle, 2006).

Rumen acidosis is a common problem under feedlot conditions where cattle change from a roughage-based to a high concentrate-based diet (Owens et al., 1998). It is associated with an imbalance between lactic acid producing and lactic acid utilizing bacteria (Bach et al., 2007). *Megasphaera eldenii* is an important lactic acid utilizing bacteria which can now be produced for commercial use and supplemented for the prevention of lactic acid build up in the rumen and subsequent acidosis. As stated, an adaptation period is necessary for the rumen microbe populations to adapt to the new diet where the inclusion of non structural carbohydrates (NSC) increases over a period of a few weeks until the so called 'top' diet is reached with a minimum of roughage. The adaptation period is

required in order for numbers of lactate utilizers such as *M. elsdenii* to increase to levels effective against subsequent build up of lactic acid. Cattle that enter the feedlot and are dosed with sufficient levels of *M. elsdenii* could theoretically be put straight on to the 'top' diet without adaptation to the diet change.

The purpose of this trial is to identify the effects of a strain, NCIMB 41125 (MeCH4), of *Megasphaera elsdenii* on the adaptation period of feedlot cattle on diets formulated for high starch levels. MeCH4 was isolated from the rumen of cattle adapted to high starch diets. By converting lactic acid in the rumen to volatile fatty acids (VFA's) such as butyrate, *M. elsdenii* should be able to at least reduce the adaptation phase, if not totally eliminate the need for it without effects on intake patterns, feed conversion rates, health and carcass yield. These statements are however based on the assumption that MeCH4 is the only limiting step in the adaptation of the rumen to high starch diets. If results are positive, this strain of *M. eldenii* will have great economic implications to all feedlot farmers. The following chapter will delve into the rumen environment with relative depth and highlight the constraints faced when feeding the ruminant with aims of optimizing productivity.

Chapter 2: Literature Review: The rumen environment

2.1) The rumen environment and constraints

In order for ruminants to be able to survive and compete in a variety of different environments based on the utilization of a variety of different feedstuffs, they require a rumen ecosystem which is diverse enough to cope with the above variables (Kamra, 2005). They have therefore adapted a rumen ecosystem with a highly diverse microbial population capable of doing so. Due to the sheer numbers of ruminal microbial groups and their interaction it is very difficult to determine the role played by a particular group in the rumen (Kamra, 2005). The end result of their actions is however what is most important, specifically their ability to convert high fiber feeds, which is otherwise useless to the animal, into a source of energy which can be utilized by the animal is what makes them of such value (Kamra, 2005). The primary purpose of the rumen fermentableprocess, therefore, is to present the necessary nutrients for microbial growth (Chalupa, 1997). It is the present rumen environment constraints which aids in the maintenance of a stable rumen environment and prevents the invasion by outside microbes. These environmental constraints are either natural or feed associated and will be responsible for the limitation of growth of the microbes entering the rumen mainly through feed, drinking water and air (Kamra, 2005).

Listed below are the major environmental constraints (Kamra, 2005):

- Anaerobiosis
- High buffering capacity

- Osmotic pressure
- Saprophytic competition between microbes

The anaerobic conditions in the rumen are essential for survival of the microbes even though they are able to tolerate minimal levels of oxygen upon exposure (Kamra, 2005). This however serves as the major method of preventing invasion by foreign microbes and subsequent disruption of an otherwise stable environment. The environment is maintained by gasses released as by-products of microbial fermentation, namely carbon dioxide, methane and hydrogen (Kamra, 2005). The high buffering capacity and osmotic pressure also has detrimental effects on microbes entering the rumen from outside and some established microbes even have the ability to prevent the survival of foreign microbes by producing anti-microbial compounds (Kamra, 2005). Feed-associated constraints are generally associated with anti-nutritional compounds which are produced by plants in an attempt to protect themselves against invading microbes. When these plants are consumed by animals, these compounds, namely tannins, lignins, saponins and mimosine inhibit the growth of a variety of different microbes (Kamra, 2005).

2.2) Microbial diversity

The microbes inhabiting the rumen consist of bacteria, protozoa and fungi with bacteria and protozoa being the most important (Moran, 2005). Up to 80% of the digestible dry matter in the rumen is digested by bacteria with different species being capable of digesting for example starch and cellulose. The source or composition of the animal's diet determines the numbers and proportions of the three groups of microbes due to the

fact that the end products of fermentation are the substrates for microbial cell growth and proliferation (Moran, 2005). The maintenance of a healthy mix of bacteria and protozoa is essential to allow the rumen to continue to function efficiently (Moran, 2005).

Bacteria

Bacteria are commonly found at three major sites in the rumen and are classified accordingly (Baldwin & Allison, 1983). The first and most abundant consists of those microbes which are detached from plant matter and are suspended in rumen fluid. The second is comprised of those bacteria which are firmly attached to or within the feed particles. A third is represented by those bacteria which attach to the reticulo-rumen mucosa (Baldwin & Allison, 1983). Although most of the bacterial species have been identified there still remain numerous populations which are still being identified (Bauchop, 1979).

Common features of bacteria isolated from roughage fed cattle are as follows (Kamra, 2005):

- Gram-negative bacteria dominate with gram-positive numbers increasing on higher energy diets.
- Most are obligate anaerobes.
- They have an optimum pH range of 6.0 – 6.9.
- And an optimum temperature of 39 degrees Celsius.

Protozoa

The number of protozoa inhabiting the rumen is far lower than that of bacteria (Baldwin & Allison, 1983). However due to their greater size, they are able to compensate for their relatively low numbers and still make up approximately 50 % of the total rumen microbial mass (Bryant, 1977). Ciliate protozoa have been grouped according to their morphological characteristics. They therefore fall into one of the two following groups, namely holotrich or entodiniomorphid protozoa (Kamra, 2005).

Defaunation of protozoa due to disruption of the rumen environment in some way may have a number of effects on animal performance, namely (Kamra, 2005):

- Lack of pH stabilization with a low rumen pH occurring.
- Lactic and propionic acid build up in rumen liquor.
- Ammonia nitrate decrease.
- Reduction of methanogenesis.
- Rapid increase in the number of bacteria and fungi present in the rumen liquor.
- Increased feed conversion rate of high roughage diets due probably to the removal of a function of protozoa on the above mentioned diets.

Fungi

Obligate anaerobic fungi that reside in the rumen as well as other regions of the digestive tract play a significant role in the digestion of fiber (Kamra, 2005). Proof of this is given by the presence of enzymes in fungi that are required for the effective break down of fiber (Williams & Orpin, 1987). Fungi in the rumen are known to preferentially bind to the more rigid cellulosic tissue of fiber using rhizoids to penetrate the cell walls allowing

extensive breakdown by enzymes from fungi and other microbes residing in the rumen (Akin, 1986). For slow growing organisms such as fungi, which have a longer generation time than bacteria and protozoa, fibrous particles must remain in the rumen for a relatively long period to ensure effective digestion (McAllister et al., 1994).

Fungi have a generation time of between 24 – 30 hours as opposed to protozoa with a generation time of between 5 and 14 hours (Bauchop, 1981). Populations of these microbes will therefore decrease rapidly if particles increase passage through the digestive tract as is the case with pelleted diets which have shorter transit times than unprocessed feed and will not support the growth of anaerobic fungi (Kamra, 2005). Therefore fiber residence time in the rumen is the most important factor determining the effectiveness of fungal species. This idea was supported by trials where the addition of *A. Oryzae* had no effect on fiber digestion or nitrogen balance in situations where residence time was too short (Niver et al., 1973).

2.3) Rumen degradation and fermentation

The primary source of nutrient required for microbial growth and maintenance is provided by fermented substrates (Hungate, 1966). These substrates are provided by the processes of digestion and fermentation of food ingested and exposed to rumen microbes (Chalupa, 1977). The nutrients in the food eaten by cattle are generally made up by three distinct groups. These are the carbohydrates, proteins and the fats or lipids (Moran, 2005). Approximately 75% of the dry matter of plant tissue is in the form of

carbohydrates which therefore makes the largest contribution to nutrient provision to microbes (Moran, 2005). The rate at which feed is digested is dependent on a number of factors, namely the quality and composition of the feed, the number and types of microbes present, rumen pH as well as the nutrients that may be limiting in the rumen and the removal of microbes from the rumen (Moran, 2005).

2.4) End products of fermentation

The major end products of microbial fermentation are as follows (Moran, 2005):

- Volatile fatty acids
- Ammonia which is used by bacteria for protein production
- Carbon dioxide and methane which is either removed through the rumen wall or via erucation.

The three major volatile fatty acids produced are acetic, propionic and butyric acid. The proportions of which are determined mainly by the composition of the diet (Moran, 2005). Acetic acid production dominates during the fermentation of fiber with a high acetic : propionic acid ratio being experienced in cattle fed a high fiber, low energy diet (Moran, 2005). Propionic acid production on the other hand increase steadily with starch and sugar fermentation, with a low acetic: propionic acid ratio in most cases being associated with feeds high in rapidly fermentable carbohydrates (RFC) such as cereal grains (Moran, 2005). Small amounts of lactic and succinic acid production is expected from carbohydrate fermentation when fiber is the main source of carbohydrates in the diet (Hobson, 1972). These levels however increase when cereal grains become the major source of carbohydrates with a rapid increase in levels of rapidly fermentable

carbohydrates (Hobson, 1972). The increase in lactic acid is however proportionately far higher than that of succinic acid.

These volatile fatty acids (acetic, propionic and butyric acid) are the source of energy required to produce microbial protein from amino acids and ammonia produced as the end product of protein degradation in the rumen (Hobson, 1972). Microbial protein is made available to the animal from microbes washed out of the rumen by digestion in the small abomasum and lower digestive tract (Hobson, 1972).

Although fats should not be included at levels higher than 5% of dietary dry matter, they still act as an important alternative source of energy to the micro-organisms in the rumen (Hobson, 1972). The reason for the low inclusion rate of fat is due to its negative influence on cellulose digestion therefore limiting its benefit in high-roughage diets (Davison & Woods, 1960). The reason for this being its ability to coat fiber particles and therefore reduce access by micro-organisms preventing subsequent fermentation. These negative effects are however reduced in low-fiber diets but inclusion is still limited to 5% as fat feeding also causes increase in carcass back fat as well as liver fat (Buchanan-Smith et al., 1974).

In commercial feedlots, beef cattle are gradually adapted to a final low fiber high grain diet. This adaptation process is designed to minimize the incidence and risk of acidosis. In practice however, cattle in large feedlots are fed in pens with wide swings in intake by individual animals. This transition adaptation period, therefore represents the time when acidosis is most likely to occur (Galyean, 2001). Reliable data on the incidence and

economic impact of acidosis in the South African feedlot industry seems scarce, data from the USA, however, shows that digestive disorders (of which acidosis forms the major part) account for approximately 25-33% of deaths in feedlot cattle, costing the feedlot industry hundreds of millions of dollars annually (Henning, 2004). The mechanism and symptoms of ruminal acidosis, prevention thereof and the impact on the feedlot industry will be discussed in chapter 3.

Chapter 3: Rumen acidosis: Mechanism, Prevention and implications for the feedlot industry

3.1) Introduction

The majority of losses in production of feedlot cattle associated with both mortality and morbidity can be attributed to digestive disturbances and respiratory diseases with ruminal acidosis being most likely the most important digestive disturbance (Nagaraja & Lechtenberg, 2007). It is also regarded as the most common digestive upset in the dairy industry, having major effects on production with regards to milk composition and milk yield due to an associated drop in feed intake (Bach et al., 2007). Other common effects include diarrhea, laminitis and liver abscesses, all of which may effect milk production of these animals. Rumen pH is a very important factor which determines the functioning and stability of the rumen (Nagaraja and Titgemeyert, 2007).

3.2) Acute and sub-acute acidosis

Ruminal acidosis can be classified as either subacute or acute based on the severity of the drop in pH. A Rumen pH of 5.6 or lower is in most cases considered the bench mark for acidosis. With regards to the severity of the condition, a pH range of 5.0-5.5 is regarded as subacute and a pH below 5.0 as acute (Nagaraja et al., 2007). Acute acidosis can be diagnosed by the physiological appearance of the animal for example with subsequent cardiovascular and respiratory illnesses arising which if left untreated could result in death (Huber, 1976). Other signs such as laminitis can often also be an indication that an animal is suffering from acute acidosis. As opposed to acute acidosis, subacute ruminal

acidosis is diagnosed based on losses in production or secondary signs such as a drop in dry matter intake (DMI) or average daily gain (ADG) rather than direct signals of illness. Other signs such as liver abscesses and rumen ulcers can often be observed at the abattoir which unfortunately is too late for cows that were already suffering from the condition (Bach et al., 2007).

3.3) Mechanism of rumen acidosis

The build up fermentation end products, namely lactic acid as well as other volatile fatty acids (VFA's), in the rumen as a result of excessive fermentation of high concentrate diets along with inefficient removal of these acids is believed to be the major contributing factors to the manifestation of acidosis (Bach et al., 2007). Anaerobic rumen microbes produce these volatile fatty acids and lactic acid as a result of carbohydrate fermentation (Owens et al., 1998). It is the low associated pKa values of these acids and their build up which is responsible for the drop in rumen pH seen after excessive fermentation of RFC takes place. The type of fatty acid which is produced and accumulates differs in its ability to reduce rumen pH. Acetate, due to its lower pKa value, is responsible for a more drastic fall in pH when compared to propionate and butyrate however these differences are relatively insignificant when compared to lactate (Bach et al., 2008). The build up of total ruminal acids and consequent drop in pH can be attributed not only to their increased production but also to a lowered absorption rate. The lowered absorption rate means that lower levels of bicarbonate are infused into the rumen from the blood stream (Owens et al., 1998). Bicarbonate ions have a buffering capacity in the rumen and their decreased concentration will contribute to the pH fall (Owens et al., 1998). Lactic acid is known to

have a more severe effect on the pH due to its considerably lower pKa value in comparison to other acids present in the rumen (Owens et al., 1998). The production of these acids is a result of the fermentation of sugars and starch by bacteria which are present in the rumen. These bacteria can be classified as either “lactate producers” or “lactate utilizers” based on their beginning and end products of fermentation. The determining factor of whether these acids will accumulate or not is based upon the balance between these two groups (Owens et al., 1998).

Under normal grazing conditions where levels of total fiber and physically effective fiber in the diet are relatively high, the accumulation of these acids, especially lactic acid is low and will not be sufficient to reduce rumen pH too severely as to cause any digestive disturbances due to the absorption and metabolism of these acids by body tissues (Owens et al., 1998). However, in cases such as the situation in the feedlot and the TMR dairy industry, where sufficient energy must be provided to sustain high levels of production, the proportion of fiber to non fiber carbohydrates in the rations must be decreased in order to increase the readily available energy source of the residing microbes. This change in ration composition is believed to trigger a potentially acidogenic situation (Bach et al., 2007). Simultaneously though, these concentrate diets also provide more energy for rumen fermentation (Bach et al., 2008).

3.4) Secondary conditions associated with sub-acute acidosis

Other than the obvious losses in the feedlot situation coupled to mortality under conditions of acute ruminal acidosis, losses in the form of lowered production are also

severe in cattle suffering from the subacute form of this condition. These losses are mostly attributed to the adaptation feeding phase where the animals are removed from the veld, where grazing forms the majority source of energy for rumen fermentation, and put into a situation where the majority of the fiber in their diet is substituted by concentrates in the form of feed grains (Nagaraja & Lechtenberg, 2007). Cattle suffering from acidosis during this period often show signs associated with anorexia, cyclic feed intake, diarrhea and lethargy (Owens et al., 1998). Similar studies with lambs have yielded congruent results (Krehbiel et al., 1995). The losses attributed to the above secondary conditions could prove very costly to farmers in an industry where profit margins are already minute due to sharp increases in feed prices especially that of maize and soya in recent years (more common to the USA industry) which form the bulk of the non structural carbohydrate source in the diet of feedlot cattle. Although these prices have come down recently, the effect they have on the local feedlot industry can not be ignored.

The most significant effect of subacute ruminal acidosis in a feedlot situation is its effect on production via cyclic feed intake patterns. The cause of the depression in intake is believed to be controlled by osmolality driven receptors located in the ruminal wall (Bach et al., 2007). Rumen osmolality is negatively correlated with rumen pH. Therefore as the pH in the reticulo-rumen decreases, rumen osmolality increases. Volatile fatty acid accumulation and release of cell contents from bacterial cell residing in the rumen due to cell lysis upon exposure to the low pH bring about this increase in osmolality. The lysis of these Gram negative bacteria also result in the build up of lipopolysaccharides in the rumen (Nagaraja et al., 1978). This stimulates cessation of feeding due to loss of appetite

but allows the environment in the rumen to return to normal facilitating a decrease in osmalality and the animal's appetite once again returns (Bach et al., 2007).

With individually caged animals, this mechanism of cyclic feeding can be seen with relative ease, however under normal conditions such as seen in most commercial feedlots, this observation is difficult as a group average will not be indicative of this situation (Bach et al., 2007). In such situations, a respiratory rate increase can be used as a method of diagnosis. The animal increases its respiratory rate in an attempt to nullify metabolic acidosis by getting rid of excessive carbon dioxide (Nagaraja & Lechtenberg, 2007).

In dairy cattle is has been shown that acidosis can be induced in the absence of concentrates and instead where cattle are fed alfalfa pellets (Plaizier et al., 2007). Results of these studies show that effects on feed intake differ for subacute ruminal acidosis induced by grain feeding as opposed to alfalfa pellet feeding. This challenges the theory that fiber digestion, volatile fatty acid production and rumen osmolality are the only factors causing a drop in intake (Plaizier et al., 2007).It is believed that inflammation of various internal organs of cattle can be another contributing factor to the subsequent drop in feed intake (Plaizier et al., 2007). Inflammation of the epithelial tissue of the rumen wall may present an example of this (Bach et al., 2008).

Apart from the effect of acidosis on fiber (Beauchemin, 2000) and protein digestion (Bach et al., 2005), the associated pH changes the population of microbes in the rumen (Bach et al., 2008). What makes matters worse is the fact that the lactic acid producing

species of bacteria in the rumen such as the *Lactobacillus* sp and *S.bovis* are more tolerant to low pH levels than other lactic acid utilizing species or fibrolitic bacteria. This allows these species, especially *Lactobacillus* sp, to proliferate even at very low pH's (Nagaraja et al., 2007). Rumen bacteria are however not the only microbes responsible for the production of lactic acid. Rumen protozoal species such as the *Holotrichs*, *Isotricha* and *Dasytricha*, account for a large amount of lactic acid produced in cattle that are consuming diets high in grains (Nagaraja & Titgemeyer, 2007). However ciliated protozoa numbers in the rumen and lactic acid production seem to be inversely related due to their predatory nature towards bacteria. They are therefore able to slow lactate production by reducing bacterial sugar and starch fermentation through their effects on bacterial population numbers. This might help to buffer pH changes in the period directly following feeding (Nagaraja & Titgemeyer, 2007).

3.5) **Methods of prevention**

Three main strategies are proposed in the prevention of ruminal acidosis (Bach et al., 2008):

a) Dietary roughage
b) Feed bunk management
c) Use of feed additives

3.5.1) Ration formulation

The inclusion of a minimum amount of total fiber and physically effective fiber is in general the most common attempt to negate acidosis (Bach et al., 2008). Cattle fed high-grain diets such as those in the feedlot situation produce only 60 to 70 percent of the saliva when compared to cattle that have high levels of roughage intake (Schwartzkopf-Genswein et al., 2003). It has however been shown through research that the proportion of neutral detergent fiber (NDF) in the diet accounts for only 50 percent of pH variation and that NSC's are responsible for the remainder of the variation (Bach et al., 2008). It seems most likely that this decrease in NSC due to higher inclusions of fiber aids in the prevention of subacute rumen acidosis (Bach et al., 2008). The inclusion of elevated levels of physically effective fiber is proposed to aid in the ultimate buffering capacity of the rumen through stimulation of increased chewing times and higher saliva production and secretion. An increase in particle size of forages has also been proposed as a successful method for preventing acidosis (Bach et al., 2008). This was confirmed with studies on alfalfa hay inclusion in total mixed rations (TMR) where larger particle sizes resulted in linear increases in chewing as well as rumination times (Clark and Armentano, 2002). The definition of physically effective fiber refers to the proportion dietary neutral detergent fiber contained in particles above a specified particle length (Bach et al., 2008). This definition has however led to a large amount of variation as values for the specified length differ widely (Bach et al., 2008). Mertens (1997) proposed this length to be above 1.8 mm, differing from the particle lengths of above 8 and 19 mm suggested by (Lammers at al., 1996). Due to these discrepancies between values, it makes suggesting a unique physically effective fiber value to prevent sub-acute rumen acidosis very difficult (Bach et al., 2008). The proposed relationship between physically effective fiber and

rumen pH also tends to be inconclusive. Some studies have revealed that indeed a relationship does exist (Soita et al., 2000; Calberry et al., 2003). Others however indicate the contrary (Yang et al., 2001; Kononoff et al., 2003).

Studies have revealed that an increase in forage particle size has not always resulted in the expected pH rise or reduced sub-acute rumen acidosis incidence if selective sorting against these larger particles occurs (Bach et al., 2008). If particle size is increased to above 27mm, animals may feed selectively and thereby sort out concentrates from longer fiber particles and still show signs of sub-acute rumen acidosis (Leonardi et al., 2005). It is therefore essential that fiber particles included in the TMR be within a specified range to be effective in combating rumen acidosis (Bach et al., 2008).

3.5.2) Feed bunk management

The major role of bunk management in the prevention of subacute rumen acidosis is in the elimination of variability in intake (Schwartzkopf-Genswein et al., 2003). The following practices were proposed by (Owens et al., 1998) and (Schwartzkopf-Genswein et al., 2003):

- Reduction of meal size
- Programmed feeding
- Multiple feed deliveries per day
- Timed feeding

The use of the above management practices has been to stabilize feeding of cattle in the feedlot thereby reducing daily variation which could lead to the manifestation of digestive disorders such as subacute rumen acidosis (Schwartzkopf-Genswein et al., 2003). While most literature supports this, not all literature supports the view of restricted feeding being used as a management tool against subacute rumen acidosis. Various studies have revealed that animals under such feeding regimes become “meal eaters” and the consumption of a few large meals per day may lead to increased rumen pH variability (Zinn, 1995). This variability seems to be mostly attributed to an increased rate of intake upon presentation of a meal (Fanning et al., 1999).

3.5.3) Use of feed additives

The following list of direct fed microbials and feed additives are continually made use of in the prevention and treatment of acidosis in ruminants (Bach et al., 2008):

- Ionophores
- Bacteria
- Fungi
- Organic acids
- Yeasts
- Buffers and alkalinizers

A wide variety of these additives are fed routinely in dairy cattle diets, but due to very low profit margins in feedlots, most of these additives are fed to a limited extent in feedlots. Ionophores are probably the only additive that is currently being fed as a standard in South African feedlots. Negative public perceptions on the use of "hormones and antibiotic additives", however has forced the feedlot industry to revisit their feeding strategies and urgently investigate the potential of more "natural" feed additives such as direct fed microbials.

3.6) Implications for the feedlot industry

Acidosis in feedlot cattle is a result of exposure of calves to final high concentrate diets too quickly (Taylor, 1994). On the other hand, studies with dairy cattle indicate that a great amount of variability exists with regards to acidosis incidence rates (Beauchemin, 2007). Various factors have been noted as responsible for this variation including eating rate, sorting of feed, salivation rate etc (Garret et al., 1999). Direct prevention would insist a longer adaptation period where the cattle are exposed to higher concentrate: roughage ratios more slowly to allow the rumen microbe populations to make the necessary adjustment to the alteration in diets (Taylor, 1994). However this would mean losses in the form of lowered production from the animal's potential to grow when fed these high energy concentrate diets. Therefore the aim of feedlot managers should be not only to reduce the incidences of ruminal acidosis, both acute and subacute, but also to shorten the period required for the rumen microbe population to adapt to the increased supply of fermentation energy in the form of NSC's, sugars and starches. This will

therefore reduce the number of days required to feed cattle to slaughter and improve the margins in an already difficult industry. One possible proposed solution to shorten the adaptation phase would be the supplementation of direct fed microbial's in ruminant diets to combat acidosis and associated losses in production (Krehbiel et al., 2006).

The effects of ionophores and more "natural" feed additives such as yeast products and other direct fed microbials, in particular *Megasphaera elsdenii*, will be discussed in more detail in Chapter 4.

Chapter 4: Feed additives in ruminant nutrition: Ionophores, direct-fed microbials, yeast products and *Megasphaera elsdenii.*

4.1) Direct-fed microbials

Direct fed microbials (DFM's) or probiotics are fed to livestock as supplements with the aim of improving the health and performance of the animals (Yoon et al., 1995). They include bacteria, fungi and yeasts and are available in a number of forms such as powders, pastes, boluses and capsules (Garnsworthy and Wiseman, 2001). Direct fed microbials may also be mixed and administered in feed or drinking water but many researchers are skeptical about the effectiveness of such administrations. Most microbes are not tolerant of high heat levels and although exceptions such as bacilli species exist, heat processing during pelleting of feeds would destroy the majority of direct fed microbials (Garnsworthy and Wiseman, 2001). The general idea of ruminant inoculation with microbes which are believed to be beneficial is not a new concept as the inoculation of sick animals with fluid of rumen origin from healthy animals has routinely been practiced (Garnsworthy and Wiseman, 2001). Their use has increased though due to the increased demand for 'natural' growth-promoting substances (Kung Jr, 1999).

Proposed primary actions of direct fed microbials are as follows: (Krehbiel et al., 2006):

- Reduce the growth of pathogenic bacteria.
- An increase in the numbers of desirable microbe in the gut.
- Facilitate the digestion of fiber.
- Inactivate toxins

The above actions can result in useful benefits for the feedlot industry with associated increases in average daily gain and improved feed efficiency (Krehbiel et al., 2003). These additives may also be used to reduce the potential incidence of problems such as ruminal acidosis (Krehbiel et al., 2003). Newly arrived beef calves which enter the feedlot have been exposed to a number of stresses such as recent weaning, fasting, transport, vaccination, castration and dehorning (Krehbiel et al., 2003). Stress in these forms may and probably will disrupt rumen microbe populations. Providing them with direct fed microbials shortly after arrival may reduce the associated losses in production by repopulating the gut with desirable microbe populations (Krehbiel et al., 2003).

Direct fed microbials exist in two basic forms based on their origin. These include bacterial direct fed microbials (DFM's) and fungal DFM's (Kung Jr, 1999). Not all DFM's carry out their action in the rumen. Most bacterial-based DFM's have an effect on the lower tract such as *Lactobacillus acidophilus*, which is a lactic acid producing bacteria (Kung Jr, 1999). It has a beneficial effect in the small intestine where it lowers the pH due to lactic acid build-up and thereby inhibits the growth of pathogenic microbes. Fungal DFM's have also been popular additions to ruminant diets in the past and occur in three main forms (Kung Jr, 1999):

- Those that contain and guarantee 'live' yeast.
- Secondly additives which contain *Saccharomyces cerevisiae* and culture extracts with no guarantee of live organisms.
- Lastly those fungal additives based on Aspergillus oryzae fermentation end products with also no guarantee of live organisms.

Fungal DFM's facilitate beneficial changes in rumen activity by their stimulation of growth of rumen protozoa and their action will be discussed further under yeast supplementation (Kung Jr, 1999).

However, for direct fed microbials to be effective they must meet certain criteria (Krehbiel et al., 2003). They must not be pathogenic towards the host, must be able to survive in the specific regions of the digestive tract and not be liable to actions of either saliva, gastric juices or bile, must be genetically stable and be host specific (Krehbiel et al., 2003). If indeed they meet the above criteria, they aught to be able to carry out desired actions by outcompeting pathogenic bacteria for attachment, facilitating of antibacterial effects or eliciting an immune response, thereby maintaining a safe environment for the proliferation of desirable microbes (Krehbiel et al., 2003). Responses to microbial feed additives are often negligable and variable which can make research very frustrating, but with current research undertaken to define the specific mode of action of these additives, more reliable activities can be devised (Newbold et al., 1992). The effects of ionophores as well as two DFM's, namely yeast products and *Megasphaera elsdenii* will now be discussed in more detail.

4.2) Ionophores

The use of ionophores to increase performance and control bloat and coccidiosis in feedlot cattle is not a new concept (Krehbiel et al., 2006). Ionophores act by altering the rumen microbial populations and subsequently the ratios of VFA's produced (Krehbiel et

al., 2006). Ionophores are toxic to many higher organisms including protozoa, bacteria and fungi (Russel & Strobel, 1989). The toxicity of ionophores is attributed to their ability to penetrate cell membranes and alter ionic fluxes to and from the cell (Ipharraguerre and Clark, 2003). Gram-positive bacteria are known to be more susceptible to destruction by ionophores than gram-negative due to their less complex cell membrane structure (Russel, 1996). Gram-positive bacteria are involved in the production of lactate, acetate, butyrate and formate amongst others (Ipharraguerre and Clark, 2003). As previously stated, an excessive production of lactate can result in acidosis if the rumen is not adapted to this increase which is generally associated with feeds of low fiber and high NSC levels (Nocek, 1997). Therefore ionophores carry out their net positive effect of reducing lactic acid levels by eliminating the bacteria that produce it. On the other hand, bacteria which are associated with the production of propionate and succinate as well as those which are responsible for lactate utilization are not sensitive to ionophores (Bergen & Bates, 1984). The insensitivity of *M. elsdenii* to ionophores such as monensin means that the two additives can safely be fed together without adverse effects on one another in the rumen environment (Ipharraguerre and Clark, 2003). The added advantage of a higher propionate : acetate ratio and therefore an improved energy balance justifies the inclusion of ionophores in the TMR's of feedlot cattle (Burrin and Britton, 1986).

4.3) Yeast products

The use of yeast products as feed additives is not a new practice and although the exact method of improvement in animal performance is not known, a number of mechanisms

are suggested. The supplementation of yeast has been shown to increase cellulose degradation in vitro, increase dry matter intake, average daily gain and feed efficiency in feedlot cattle (Krehbiel et al., 2006). Yeast supplementation in the form of *Saccharomyces cerevisiae* or *Aspergillus oryzae* have been known to have a stimulatory effect on the growth of lactic acid utilizing species such as *M. elsdenii* (Waldrip & Martin, 1993). They therefore have an indirect effect on the metabolism of lactic acid and the resultant increase in rumen pH (Kung Jr, 1999). The resultant increase in ruminal pH may be the reason that yeast supplementation stimulated an increase in the number of cellulolytic bacteria and therefore improved fiber digestion (Arambel et al., 1987). *Saccharomyces cerevisiae* prevented the accumulation of lactate by outcompeting *Streptococcus bovis* for glucose (Garnsworthy and wiseman, 2001). It has also been known to scavange oxygen from the rumen, creating an environment more acceptable to rumen anaerobic bacterial species (Newbold et al., 1996). The fact that yeasts have a mechanism of rumen lacate reduction so different to that of ionophores suggests that the two feed additives may well have a complementary effect (Dawson et al., 1990). Yeasts stimulate bacterial species which are not inhibited or eliminated by ionophores such as monensin (Dawson et al., 1990). Research results suggest that the supplementation of yeasts as a feed additive still has potential in both beef and dairy production systems.

4.4) *Megasphaera elsdenii*

4.4.1) General

Megasphaera elsdenii resides in the rumens of all ruminants as well as cattle which are fed a high-grain diet in which lactate is important. This was substantiated by cell counts that were obtained from young calves, in which lactate fermenting cocci of the order of $1x10^9$ CFU/mL were obtained (Hobson et al., 1958). Cell counts from mature animals did not however agree with this information. The most common explanation for the above observation on cell counts was deemed to be due to the wide variation in diet composition consumed by young and mature animals (Leedle, 2006). Young calves had diets composed mainly of milk which means higher levels of lactose intake. The absence of lactose, with glucose as a major degradation intermediate, from the diets of older animals meant that the numbers of lactate fermenters such as *Megasphaera elsdenii* would be lower (Leedle, 2006). This potentially posed a problem in older cattle feeding on high grain diets, but with the onset of sound dosing techniques, risks of digestive upsets have been drastically reduced (Leedle, 2006). This strict anaerobe is a gram-negative bacteria, although it was initially believed to be gram-positive (Leedle, 2006). *Megasphaera elsdenii* was initially incorrectly named *Peptostreptococcus elsdenii* due to its association with a similar gram-positive bacteria found in the rumen of cattle experiencing bloat (Gutierrez et al. 1959). It was not until 1971 that these isolated bacteria were shown to in fact be gram-negative and therefore not actually part of the genus *Peptostreptococcus*. The bacterium was subsequently transferred to the

Megasphaera genus to form what we now know as *Megasphaera elsdenii* (Rogosa, 1971).

Cells can generally be found in pairs or chains with individual cell diameters ranging from 2.5- 3.0 µm (Leedle, 2006). For almost three decades, *M. elsdenii* was the only species in the genus and it was not until 1985 that the creation of new species was suggested such as *M. cerevisiae*, found from brewery isolates (Engelmann and Weis, 1985). It was not until the beginning of the 21st century that a number of other species were added to the genus. It was the bacteria's unusual ability to convert both D (+) and L (-) lactic acid to acetic, propionic butyric and valeric acid which interested workers at Kemira Phosphates, to investigate the potential of *M. elsdenii* to reduce the incidence and severity of bloat and rumen acidosis in cattle fed high concentrate diets (Leedle, 2006). However this seems to not be the only positive effect associated with the presence of *Megasphaera elsdenii*. Kim et al (2002) attributed the production of conjugated linoleic acid, a healthy lipid, , with the bacteria. Yanke et al (1998) commented on its ability to express phytase activity which allowed it to assist in the control of phosphates in the rumen. Lastly, Kalachniuk et al (1994) attributed the ability to lower redox potential in the digestive tract by lowering nitrate levels to *Megasphaera elsdenii* which in turn helped prevent pathogens from becoming active in the intestines.

Its interaction with *Streptococcus bovis* is also of major importance to its success. *Streptococcus bovis* responds to the relatively high levels of glucose in the ruminal fluid by growing rapidly as well as producing L-lactic acid (Leedle, 2006). If this acid were to

accumulate in the rumen it would cause a severe drop in rumen pH but even though *S. bovis* has a low affinity for glucose much the same as *M. elsdenii*, it is still able to double its numbers within 9 minutes under favorable conditions (Russel and Robinson, 1984). *M. elsdenii's* ability to use the L-lactate produced by *S. bovis* means that both bacteria play pivotal roles in the maintenance of a stable rumen environment (Leedle, 2006). Considering its biochemistry and metabolism it seems to be rather limited in its activity with its activity being restricted to the fermentation of a few carbohydrates as well as lactic acid (Leedle, 2006).

4.4.2) Substrate usage

It is common knowledge that numbers of *M. elsdenii* increase with the onset of high levels of grain feeding (Mackie and Gilchrist, 1979). The rate of increase is relative to the original numbers of the bacteria prior to the onset of grain feeding. The numbers however are low in cattle grazing on veld which supports the hypothesis that dosing the bacteria shortly prior to the onset of concentrate feeding would drastically shorten the period required for the bacteria to establish themselves in the rumen and be effective in maintaining the lactic acid levels below that which could cause digestive upsets for the animal (Leedle, 2006). Slyter, 1976, proposed that the success of *M. elsdenii* could be attributed to its ability to use lactic acid and its quick increase in numbers. He found that *M. elsdenii* numbers in the rumen of sheep increased 21-fold after they were changed to a diet of hay and corn as opposed to *Selenomonas ruminantium* numbers which increased only 3.8-fold. The pathway by which the bacteria converts lactic acid to substrate useful to the animal also distinguishes it from other lactic acid utilizing bacteria and contributes

to its success (Rogosa, 1984). *Megasphaera elsdenii* ferments DL-lactate, producing acetate, propionate, n-butyrate, n-valerate, n-caproate hydrogen and carbon dioxide as products of fermentation (Rogosa, 1984). When the bacteria has lactate as substrate, propionate predominates as the major end-product of fermentation while butyrate and caproate predominate when glucose is the substrate (Leedle, 2006). *Megasphaera elsdenii* in co-culture with amylolytic bacteria ferments not only lactate but is also capable of fermenting soluble sugars which arise from the degradation of starch (Marounek and Bartos, 1987).

Although *Megasphaera elsdenii* is not the only lactate utilizing bacteria in the rumen, it is however possible to determine the amount of lactate that this bacteria converts to propionate (Ladd, 1959). This is based on the fact that it is the only bacteria to use a non-randomizing pathway for the conversion of lactate to propionate with acrylate as an intermediate (Elsden et al., 1956). It was therefore later confirmed using this principle that *Megasphaera elsdenii* fermented 60 to 80 % of the DL-lactate present in the rumen of animals fed normal diets (Counotte et al., 1981). As previously stated, *Megasphaera elsdenii* is able to use either glucose or lactate as substrate of fermentation. The bacteria does however have a preference. It was shown with *Megasphaera elsdenii* strain NIAH 1102 that lactate is used preferentially to glucose when the two substrates are present together (Hino et al., 1994). This was compounded by trials where cells utilizing glucose in the absence of lactate switched to lactate when lactate was added and did not switch back to glucose until lactate levels dropped to very low concentrations (Hino et al., 1994). Co-culture experiments that investigated the effects of *Megasphaera elsdenii* and

Streptococcus bovis strain JB1 on glucose substrates yielded more interesting results. *Megasphaera elsdenii* was overgrown by *Streptococcus bovis* due to the fact that *Megasphaera elsdenii* was obligated to use the lactate produced by *Streptococcus* as substrate (Leedle, 2006). A common phenomenon, the "glucose effect" where bacteria preferentially use a particular substrate to exclude another is applicable to *Megasphaera elsdenii*. Glucose and maltose are inhibitors of sucrose utilization which could be a problem in calves who rely on sucrose as a source of energy (Russel and Baldwin, 1978). Lactate however has no such effect on sucrose utilization and no interactions are seen between glucose, maltose and lactate (Russel and Baldwin, 1978). A major question arose from recent studies with *M. elsdenii*. Why is *M. elsdenii* unable to produce propionate when glucose is the substrate of fermentation? This question was answered after an in depth look into the enzyme systems and pathways associated with the bacteria (Hino and Kuroda, 1993). The primary reason for this is that the cells fermenting glucose lack the synthesis of the enzyme lactate racemase, induced by the presence of lactate, and essential for the production of propionate (Hino and Kuroda, 1993).

4.4.3) Survivability

The generation times of *Megasphaera elsdenii* strains prior to the isolation of the strain NCIMB 41125 (CH4) were between 125 and 200 minutes on complex growth media (Forsberg, 1978). However, since the isolation of the CH4 strain it was shown that the generation time was reduced to 50 minutes when grown on a lactate media, a growth rate approximately four times faster than other isolated strains (Kemira Phosphates, 2006). A possible problem encountered by this bacteria is however its inability to withstand total

nutrient starvation (Leedle, 2006). Were *Megasphaera elsdenii* to be totally starved of substrate, cell numbers would decrease drastically over a short period of time. Studies however indicate that this situation is almost never the case within the rumen as ruminal contents of cattle fed high-grain diets contain large amounts of lactate especially shortly after the onset of feeding of these high-grain diets. Secondly with the abundance of carbohydrate and lactate available to *Megasphaera elsdenii*, cells would almost certainly contain large amounts of carbon storage material identified as glycogen which would aid in the survival of these bacteria even during periods of nutritional stress (Leedle, 2006).

The effects of many important antimicrobial agents commonly used in the feedlot system such as monensin and tylosin on *Megasphaera elsdenii* CH4 were evaluated in order to determine its effectiveness as a direct fed microbial under normal feedlot processing conditions (Leedle, 2006). Although it is sensitive to penicillin G, erythromycin and chloramphenicol, all strains isolated up to date have been resistant to virginiamycin at a concentration of 50 µg/ml (Leedle, 2006). Some would consider antibiotic resistance to be a risk. The thought being that this resistance is most probably encoded by genetic elements which could possibly be transferred to indigenous bacteria with the risk that these bacteria then become resistant to antibiotics, becoming a health hazard to all consumers (Leedle, 2006). If this resistance is however due to a structural, and not a genetic element, the safety to man and animal would not be compromised (Leedle, 2006). Recent research by Kemira Phosphates on their isolated strain, CH4, has proved that it is safe to be used as a live fed direct fed microbial for ruminant animals (Leedle, 2006). Their survivability is also very dependent on the diet, and it was shown that sheep fed a

diet containing 60% or less grain and molasses had amylolytic bacteria numbers 10-fold that of the lactic acid utilizers (Mackie et al., 1978). The very low population numbers as opposed to other bacteria populations residing in the rumen are overcome by a far faster growth rate than their counterparts allowing them to respond positively to lactic acid levels in grain fed steers (Leedle, 2006).

However when the diet was changed to a 71% concentrate inclusion, the numbers of lactic acid utilizers increased by 10- to 100-fold which enabled the excess lactate produced during fermentation to be easily metabolized (Mackie et al., 1978). After 21 days of these sheep consuming the 71% concentrate diet, the numbers of amylolytic bacteria and lactate utilizers became about equal. Further studies concluded that the lactic acid consuming population must increase in number to levels at which their lactate utilization could keep up with lactate production but under conditions such as roughage feeding, there is insufficient lactic acid produced to even maintain high enough numbers of lactic acid utilizers (Mackie and Gilchrist, 1979).

4.4.4) *Megasphaera elsdenii* and rumen acidosis

Early studies revealed that for animals consuming roughage based diets, less than 1% of the ingesta is fermented by way of lactic acid and for those consuming concentrate based diets the value is less than 17% (Slyter, 1976). It was therefore initially thought that the presence of lactic acid in the rumen would not pose as great a threat as it is now proved to be. Further studies into the prevalence of rumen acidosis, both acute and sub-acute

indicated otherwise (Leedle, 2006). Later studies revealed that if the production of L-lactic acid by *S. bovis* as well as other L-lactate producing bacteria in the rumen were to be less than the amount used by *M. elsdenii* and other lactate utilizers, a stable and favorable rumen pH would be maintained (Russel et al, 1981). Rumen pH would however decline to the point where production and performance could be compromised if production rate were to exceed utilization (Russel et al, 1981). If rumen pH were to approach 5, it has been shown that amplitude and frequency of contractions would gradually decrease to a point where it would eventually cease all together (Leedle, 2006). It is the ability of *M. elsdenii*, especially the CH4 strain, to ferment L-lactate and produce volatile fatty acids at rates high enough in most cases to overshadow the production of L-lactate which makes this rumen bacteria of such value (Leedle, 2006).

The maintenance of rumen pH is linked and therefore dependent upon the balance of bacteria that reside in the rumen. Therefore a poor microbial balance could easily have a negative effect on the functioning of the rumen of the host animal (Leedle, 2006). Even though the tolerance by different rumen species to a pH drop differs, at some point nearly all are negatively affected. Except for a few of the lactic acid bacteria, most of the other species are generally neutrophilic and therefore require that their environment remains neutral or slightly alkaline (Leedle, 2006). *Fibrobacter succinogenes* are sensitive to a pH below 6.2, therefore when the pH reaches a level of 6.0 or below, cellulose digestion decreases rapidly (Leedle, 2006). It is not only the external pH that is of concern but also the bacteria's internal pH (Leedle, 2006). Although *Megasphaera elsdenii* is resistant to relatively low external pH's, the effect of a low internal pH is still not known. Even

though resistant to lower pH's, at a pH of 5.5 a decline in its growth rate and growth yield as well as an increase in the lag phase can be noticed (Leedle, 2006). It still remains a species which is more tolerant than most to drops in rumen pH. The reason for this tolerance is as yet not fully understood but current research suggests that it surrounds its ability to expel protons more readily than the more acid-sensitive bacteria (Leedle, 2006). Its tolerance may however not be due only to this capacity and other factors may be involved (Leedle, 2006). These factors include its ability to tolerate a low pHi (internal pH) as well as its ability to generate sufficient energy from rumen substrates in spite of the fact that it expends a large amount of energy during proton expulsion (Leedle, 2006).

Because of the major threat that rumen acidosis poses to both the dairy and feedlot industry, several methods of prevention or control have been evaluated in an attempt to reduce losses associated with the condition (Leedle, 2006). These methods range widely from the use of feed additives to the use of antibiotics and furthermore, the effectiveness of each also varies (Stone, 2004). Other attempts included the inoculation of sick animals with rumen fluid from animals which are assumed to be adapted or the dosing of specific bacteria which are known to ferment lactic acid such as *Megasphaera elsdenii* CH4 (Leedle, 2006). Although antibiotics such as virginiamycin and ionophores have proved effective under certain conditions, their effectiveness has not been reliable under all conditions in reducing the risk of acidosis (Thorniley, 1998). The use of antibiotics such as these may be effective but the use of natural biological alternatives to prevent lactic acid build-up still appears to be the more attractive option from a consumer and producer point of view due to the unease regarding antibiotics in meat (Leedle, 2006). This is

however not a new concept as researchers from the 1960's showed that cattle could be adapted to high-grain diets by simply inoculating them with fresh rumen fluid from previously adapted animals or from microbes adapted in a laboratory (Braun et al., 1992). These procedures were based on the assumption that the innoculi from healthy adapted animals contained sufficient concentrations of lactic acid-utilizing bacteria which once inoculated into the new animal, were able to rapidly increase in numbers to combat growing lactate levels (Leedle, 2006). It was then observed that *Megasphaera elsdenii* rapidly increased in number and established themselves in the rumen of steers which were gradually adapted to a high-energy diet (Huber, 1976). It was for this reason along with the fact that *M. elsdenii* is able to also use maltose and glucose as substrate (Russel and Baldwin, 1978) and therefore most likely out compete lactic acid producers such as *S. bovis* that allowed this organism to become one of the most likely candidates as a direct fed microbial, suitable for combating ruminal acidosis and its negative associative effects (Counotte et al., 1981).

A single pen study conducted by Klieve et al, (2003) confirmed that dosing *M. elsdenii* into the rumen of beef cattle that were rapidly switched from a forage-based diet to a grain-based diet ultimately established a viable population of *M. elsdenii* that could prevent a drop in rumen and hindgut pH. In this study, Hereford steers were individually penned and dosed with *M. elsdenii,* strains YE34 and B. Rumen fluid samples were later extracted and analyzed for pH as well as VFA, and enumerated to determine the specific bacterial populations present in the rumen. Fecal grab samples taken directly from the rectum were used for intestinal pH determination. Results from the trial indicated that

except for one steer, the lactate concentration did not rise above 2 mmol and that no other animal appeared to experience acidotic rumen or hindgut conditions. The trial also indicated that dosing was essential as *M. elsdenii* was not detectable in the steers prior to grain feeding and even though the concentration of bacteria increased rapidly after the onset of grain feeding, levels did not increase at the same rate or to the same level as dosed animals. This would affect their ability to combat acidosis.

Kung and Hession (1995) cconducted in vitro fermentation trials to test the effectiveness of *M. elsdenii* inoculation versus uninoculated cultures. For these trials, mixed cultures retrieved from the rumen of a hay-fed steer were used in conjunction with a buffer and a rapidly degradable substrate and subsequently incubated overnight with *M. elsdenii* strain B159. Results indicated that *M. elsdenii* was indeed able to prevent lactate accumulation. In cultures inoculated with *M. elsdenii*, lactate concentrations peaked at about 25 mM after a period of 5 hrs from the onset of fermentation but subsequently dropped to levels below 5 mM by 7 hrs. In untreated cultures however, lactate peaked at levels above 40 mM. It was however believed that the extent of the effectiveness of this bacteria to prevent lactate accumulation is dose dependent and this theory was supported when the dose of *M. elsdenii* was increased in the same trial with results showing that lactate levels never peaked above 2mM throughout fermentation (Kung and Hession., 1995). Other in vivo and in vitro studies confirmed results from the above study demonstrating that *M. elsdenii* could prevent lactate accumulation when a highly fermentable substrate was used (Greening et al., 1991). *M. elsdenii* significantly reduced pH as well as lactate

concentration in acidosis induced beef cattle and had the added advantage of steers inoculated with *M. elsdenii* consuming 24% more dry matter (Greening et al., 1991).

As previously stated, the balance between lactate production and consumption is only one of the many biological challenges faced by ruminants fed high-grain based diets (Leedle, 2006). It is the effect that *S. bovis* has on ruminal pH that poses a major threat to the gut ecosystem and once the critical point of overgrowth of this rumen bacteria is reached, a practical point of no return is reached even though the growth of *S. bovis* also slows dramatically due to the severely low pH (Leedle, 2006). It seem therefore utterly important that this tipping point is avoided at all costs to prevent disequilibrium. The addition of a lactate-utilizer seems to be the only answer to prevent this ultimate tipping point being reached, the reason for this being that lactate has a pKa value ten times stronger than the VFA products of lactate fermentation. Not only will this prevent such sever drops in pH but the faster absorption rates of these products will also have a positive effect on intestinal and rumen pH (Leedle, 2006).

In conclusion, from a theoretical viewpoint as well as limited in vivo/vitro studies, *Megasphaera elsdenii* appears to be an ideal candidate for use as a DFM product in an attempt at reducing and preventing lactic acid accumulation in high-grain fed steers (Leedle, 2006). Based on the fact that the livestock industry worldwide requires animals to make a speedy transition from high-forage to high-energy diets without associated problems with acidosis, a reliable and affordable solution is required (Leedle, 2006). Supplementation with *M. elsdenii* seems the most probable solution to the millions of

rands which is lost each year in both the beef and dairy industry due to excess lactic acid build-up resultion in both acute and subacute lactic acidosis (Leedle, 2006). The potential of M. elsdenii in managing intestinal and ruminal lactate levels during the adaptation phase as well as at strategic periods of the feeding cycle may prove immensely beneficial to the beef and dairy industry in South Africa and throughout the world (Leedle, 2006).

The following study (Chapter 5) is one of a series of studies funded by KK Animal Nutrition in order to research all the variables that might play a role in the successful commercial application of *Megasphaera elsdenii* in feedlot diets.

Chapter 5: Optimizing the adaptation rate of feedlot steers dosed with *Megasphaera elsdenii* and fed high starch diets: (i) Materials and methods

5.1) Introduction

Performance advantages (ADG and FCR) are the primary reasons for feedlot operators to supplement diets with DFM products. There are, however, ancillary benefits such as improved health and fewer digestive disorders which are not that easily measured (McDonald et al, 2005). Due to its affect on rumen fermentation a DFM product such as *Megasphaera elsdenii* has great potential to encourage greater feed consumption early during the adaptation phase, thereby potentially reducing the number of standing days in the feedlot. In this study the potential of *Megasphaera elsdenii,* to optimize the adaptation rate of feedlot steers, will be investigated.

5.2) Location and facilities

The trial was conducted at the Agricultural Research Council's (ARC's) experimental feedlot facility in Irene, deemed satisfactory by the ethics committee for use as research facilities for cattle. Seventy-two single pens were used for the duration of the trial. A water point was shared between two pens allowing ad lib access to fresh, clean drinking water. The single pens were approximately 2.5 x 1.5 meters in size and were sufficiently large enough as to not restrict comfort and movement of each individual animal totally. Each animal therefore had access to its own isolated feedbunk space. The pens were under roof and therefore enjoyed shade for the majority of the day also protecting the animals from the majority of other adverse whether conditions.

5.3) Experimental treatments and design

The study was carried out using a regression approach. The reason a regression approach was used was to determine a function which best fits the values of the given parameters in the trial (Altman, 1991). In most cases in this trial, a linear regression was used. A linear regression is the most basic form of regression analysis and the function is a linear equation which is used to explain how the variation in an outcome variable, Y, depends on the variation in a predictor variable, X.

However in certain situations where the outcome will depend on more than one explanatory variable such as in cases in this trial, this leads to a multiple regression. Six different starter diet roughage levels were used within each of two substrate groups namely hominy chop (chop) and ground maize that formed the major starch contributors for each of the substrate groups respectively. That resulted in a total of 12 treatments, 6 chop treatments and 6 maize treatments. The Neutral detergent fiber (NDF) level (%DM) of the diets gradually decreased from a value of 17.5% over time until a basal NDF level of 5.0% was reached on a designated day (Table 1). This decrease in NDF was facilitated by a decrease in inclusion of chop/maize starter diets with a subsequent increase in chop/maize grower diet in the mixing of the combination diets (Table 2 and Fig 1). Once each treatment reached the 5.0% NDF level, steers consumed that diet up to the end of the trial on Day 35. All the animals were kept in the trial for 35 days unless they were removed from the trial for health or humane reasons. Within each of the 12 different treatments, 6 steers were randomly allocated to each treatment (Table 1).

Table 1. Experimental layout of the study in which steers were fed 12 diets differing in starting roughage % and source of starch.

Hominy Chop based diets			
Treatment #	Starting roughage %	End roughage %	Day by which steers will reach 5% roughage
1	17.5	5.0	21
2	15.0	5.0	17
3	12.5	5.0	13
4	10.0	5.0	9
5	7.5	5.0	5
6	5.0	5.0	1
Maize based diets			
Treatment #	Starting roughage %	End roughage %	Day by which steers will reach 5% roughage
7	17.5	5.0	21
8	15.0	5.0	17
9	12.5	5.0	13
10	10.0	5.0	9
11	7.5	5.0	5
12	5.0	5.0	1

5.4) Animals

Seventy-five Bonsmara type steers were selected for the trial of which seventy-two were ultimately entered into the trial. The animals were sourced privately by Louis Hauman, a feedlot cattle buyer, and sold to KKAN and delivered directly to ARC – Irene, where the trial was conducted. Upon arrival at ARC – Irene all cattle were placed in standard feedlot group pens and received only good quality roughage and fresh, clean drinking water *ad lib* for three days prior to processing. Processing included routine feedlot vaccinations, treatment for internal and external parasites, and insertion of ear tags as well as an anabolic ear implant. Routine vaccinations and treatments included:

- Multiclos (Intervet, Schering-Plough Animal Health) for the prevention of Malignant oedema, haemorragic enterotoxaemia and pulpy kidney disease.

- Supavax (Intervet, Schering-Plough Animal Health) for prevention of anthrax, botulism and black leg.
- Bovitect PI (Intervet, Schering-Plough Animal Health) for prevention of pasteurella and infectious bovine rhinotraceheitis (IBR).
- Gardal 10% (Intervet, Schering-Plough Animal Health) for the remedy against roundworms, milk tapeworms and the liver fluke.
- Delete All (Intervet, Schering-Plough Animal Health) a ready to use pour-on for the control of ectoparasites.
- Revalor S 100's (Intervet, Schering-Plough Animal Health) an anabolic ear implant.
- Duovax (Intervet, Schering-Plough Animal Health) for the prevention of botulism and blackquarter.

On day 22 of the trial, all animals were re-vaccinated with Multiclos and Duovax and Delete All was applied for the control of ectoparasites. Two ear tags were used for identification purposes, one in each ear. The first ear tag had the animal pen number which indicated the specific single pen that the animal had been allocated to and the second showed the animal trial number along with the specific treatment that the animal had been allocated to. The ear tags were sourced from Johannesburg (SENTRAC, Jet Park, Johannesburg). Additional pest controls such as fly pesticides as well as "stride", a product sourced by STRIDE DISTRIBUTORS (ARC, Irene, Pretoria) were at hand as pests presented problems through the duration of the trial. Following processing, all seventy-five animals were weighed. The trial animals were selected according to the following criteria:

- Live-weight 200 to 250 kg
- Male - castrated (preferably) or intact
- Only Bonsmara types
- No previous exposure to concentrate feeding (apart from the veld-licks that the cows may have received)
- Healthy and free from any obvious abnormalities.

From the initial seventy-five animals, three animals were discarded because they did not meet the weight criteria as set out. The remaining seventy-two animals were then blocked according to live weight and within block allocations animals were allocated randomly to one of the 12 treatments. This random allocation of animals to the specific treatments was simply carried out by randomly selecting numbers from a hat. After processing, the steers were put into their respective single pens and were fed *ad lib* roughage until 15:00 on Day -1 after which they were fasted for 19 hours. During that time after processing which included the 19 hours of fasting, the steers had time to become accustomed to single pen conditions where they would remain for the full 35 days of the trial. Forty eight hours after processing the steers were dosed with MeCH4 (Day 1 at 08:00) and the feeding of their respective experimental diets commenced (Day 1 of trial at 10:00) signaling the end of their 19 hr fast. Within the first week, a few animals had intakes of their concentrate diets of below 2 kg due possibly to stress of adaptation to single pen conditions or lack of recognition of the diet. In such instances, 1 kg of good quality milled hay was added to their diet in an attempt to stimulate higher intake, fortunately very few such cases arose. The animals remained in the single pens for

35 days where after they were sold to Johan Cronje of Ranch Estate feedlot. Each steer was allocated 5 minutes per day during which time it was allowed to leave its pen and walk freely in a larger camp. This served as a period in which it was able to attain some exercise as well as allowing for its pen to be washed clean of faeces and urine.

The treatment of sick animals was carried out strictly according to the standard operating procedures (SOP's) below. The prescribed veterinarian, Dr. G. Catton, whom has consulting rooms at the ARC Irene Campus was on call as the need presented itself. The SOP's for sick animals were as follows:

5.5) SOP for treatment of sick feedlot calves

5.5.1) Reminders:

Meat withdrawal periods were always checked prior to injection. Date, dose, animal ID and other necessary info including the batch number were recorded upon administration.

5.5.2) Respiratory conditions:

Distinguish respiratory reactions from dusty feed reactions. The latter is more likely to occur in a large number of cattle and a history of dusty feed and conditions is obvious.

Treat respiratory infections as follows:

Day 1: Nuflor inject (Intervet, Schering-Plough Animal Health). @ 1ml/15kg intra muscular (im) and Finadyne (Intervet, Schering-Plough Animal Health) @ 2ml/45 kg intra venous (iv).

Day 3: Nuflor inject. @ 1ml/15kg im despite improvement of symptoms.

Day 5: Nuflor inject. @ 1ml/15kg im only if symptoms persist.

If not resolved consult the prescribed veterinarian.

5.5.3) Fever (>39.5°C) of unknown origin:

Day 1: Hi-tet 200 LA Gold (Bayer Animal Health (PTY) Ltd) @ 1ml/10kg im and Finadyne (Intervet, Schering-Plough Animal Health) @ 2ml/45 kg iv.

If not resolved consult the prescribed veterinarian.

5.5.4) Neurological conditions:

Symptoms include high stepping gait, weakness, seizures, hypersensitivity, "star gazing", head pressing as well as circling etc.

Day 1: Hi-tet 200 LA Gold @ 1ml/10kg im and kyrovite B1 10ml im.

If no improvement, consult the prescribed veterinarian.

5.5.5) Intestinal tract conditions:

Bloat: pass stomach tube, if signs not relieved then dose animal with 30-60 ml Bloatguard directly into the rumen. Remove the animals from trial diet and provide good quality roughage for up to 2 days. If signs persist consult the prescribed veterinarian.

Diarrhoea: Inject 10-15 ml depocillin im depending on the size and kyrovite B1 10ml im. Remove the animals from trial diet and feed good quality roughage for up to 2 days. If no improvement is seen, consult the prescribed veterinarian.

Throughout the duration of the trial, only 8 steers were temporarily removed from their single pens upon consultation with Dr. Catton. four of the steers were removed and treated for diarrhoea, with the other 4 steers being treated for Laminitis linked possibly to rumen acidosis. Seven of the steers showed immediate response to the treatment and were

returned directly to their single pens. However the steer in pen 1 was removed totally from the trial due to severe laminitis and was placed in a larger camp where it was fed hay ad lib and treatment of its condition continued.

5.6) MeCH4 application

All animals were dosed with 100ml MeCH4 at the start of the trial period. The oral dose of MeCH4 had to contain a minimum of 10^8 cfu / ml of *Megasphaera elsdenii* strain NCIMB 41125. A standard stainless steel 10L keg along with a Phillips hand dosing gun was used for the dosing of *Megasphaera elsdenii*. The timing of dosing was designed to coincide with the change from roughage to concentrate diet. The day on which animals change from roughage to the concentrate diet, and received the above-mentioned oral dose, was designated as day 1 of the feedlot trial.

MeCH4 samples were collected prior to as well as after dosing of the cattle in order for cell counts to be carried out on the samples to determine if the MeCH4 cell numbers were within specifications. The samples were analyzed at the GI-Microbial laboratory at the ARC-Irene.

For the purposes of this trial, a negative control group was in fact not included. There were two major reasons for this decision, the first being that the purpose of this trial was not to test the effectiveness of this strain of *M. elsdenii* in maintaining acceptable levels of lactic acid in order to prevent the onset of metabolic stress. This fact has been proven prior to this in other studies and the microbe has been known to effectively utilize lactic

acid, therefore potentially reducing the risk of acidosis and associated conditions at the onset of concentrate feeding (Leedle, 2006).

Secondly there were the ethical concerns surrounding the wellness of the cattle which would be subjected to such intense dietary pressure. According to Brown et al. (2006), cattle without prior adaptation to high concentrate feedlot diets, consuming diets with less than 45% roughage were liable to severe metabolic disorder and dysfunction associated with either acute or sub-acute rumen acidosis ultimately resulting in death if left untreated. With this in mind it would have been undesirable to add a negative control to the treatments.

5.7) Diets and feeding regime

Animals were fed twice per day at *ca* 08h00, just after removal of the previous day's orts and again at *ca* 15h00. Animals were fed ad lib, i.e. *ca* 10% orts left the next morning. Care was taken to ensure that no pen was without feed for any significant length of time. Orts from each pen were removed just prior to morning feeding and feed refusals were weighed daily. These orts were used to feed the three animals not included in the actual trial. Fresh clean drinking water was freely available to all animals throughout the trial. The ingredient and chemical composition of the diets and dietary adaptation regimes are shown in Table 2 and Fig 1 respectively.

Table 2. Ingredient and chemical[3] composition (% of DM) of the hominy chop and maize starter and grower diets used in the trial.

Item	Hominy Chop Starter (%)	Hominy Chop Grower (%)	Maize Starter (%)	Maize Grower (%)
Ingredients				
Eragrostis curvula	17.5	5	17.5	5
Hominy Chop	60.9	76.4	0	0
Ground maize	0	0	56.4	73.4
Wheat Bran	8	6	10	6
Multimix 40[1]	6	5	8.5	8
Molasses meal	5	5	5	5
Feed lime	1	1	1	1
Salt	0.5	0.5	0.5	0.5
Urea	1	1	1	1
Vit/min premix[2]	0.1	0.1	0.1	0.1
Chemical composition[3]				
ME (MJ/kg)	11.4	11.5	11.5	12.2
CP (%DM)	14.3	14.6	14.4	14.4
NDF (%DM)[4]	40.3	33.6	27.6	16.6
NFC (%DM)	36.9	42.5	53.5	64.7
Ca (%DM)	0.72	0.53	0.74	0.56
P (%DM)	0.60	0.63	0.41	0.38

[1]Multimix 40, a 40 % protein concentrate produced by Meadow feeds South Africa.

[2]Vitamin/Mineral premix, a blend of vitamins and minerals produced by a local premix producer, Feedmix (Jet Park, Johannesburg) with an inclusion rate of 0.2 % in the diet.

[3]Chemical composition calculated using the NRC database.

[4]NFC calculated by using the following formula: NFC = 100 – (CP + NDF + Fat + Ash).

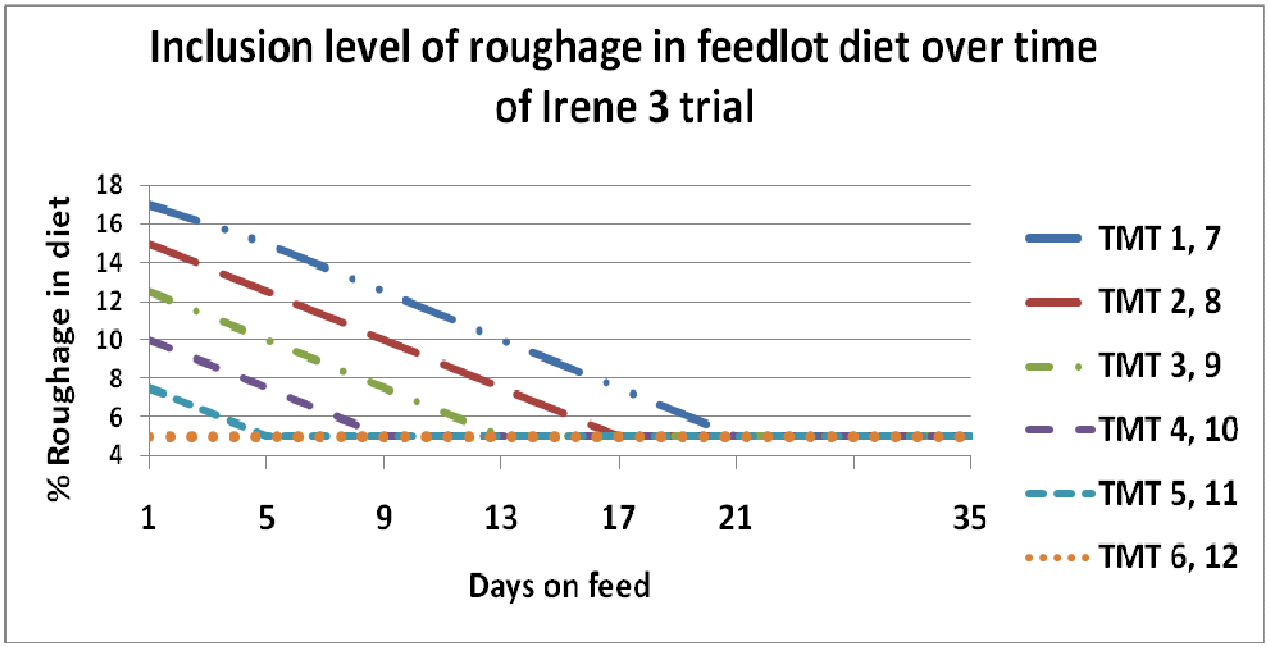

Figure 1: Diagrammatic illustration of the different roughage levels and adaptation regimes in the experimental treatments

Four basic TMR diets were mixed beforehand as well as during the trial. (Table 2).These basic diets were mixed from the raw ingredients in 1 ton batches as the need arose. From these basic diets, the 12 different combination diets (treatments) were mixed on a daily basis by mixing various ratios of the respective starter and grower diets to achieve the desired treatment diets. The objective of this feeding program design was to decrease the roughage level over time to get a more accurate indication of the interaction between MeCH4 and Roughage (Fig 1). A 280l concrete mixer (petrol) was hired from TALISMAN PLANT and TOOL HIRE, Centurion, for the mixing of these combinations. See Table A (see Addendum). Colored plastic pellets were included in small amounts (0.02% of diet) in order to make identification of combination diets more manageable. Six colors were used and each was associated with a specific chop and maize treatment group for the duration of the trial. The plastic pellets had no effect on diet palatability and

in no way affected rumen functioning. Representative samples of the 4 basic experimental diet batches (each and every one ton batch) that were mixed were collected. These samples consisted of a representative sample from pooled feed from various regions of the mixer. Dry matter (DM) was determined by drying the samples in an oven at 60ºC for 48 hours after which the samples were stored in marked plastic sample bottles. All of these samples were analyzed for DM, CP, NDF, ADF, NSC, EE and starch at UP Nutrilab, University of Pretoria. Representative samples of all the combination diets were also collected. DM was determined once again by drying in an oven at 60ºC for 48 hours and stored in marked plastic sample bottles. The samples could later be analyzed for DM, NDF and starch at UP Nutrilab, University of Pretoria, as the need arises for instance to substantiate or motivate reasons for possible unexplained results seen in the trial but were however not analyzed otherwise based on the sheer numbers and cost implications thereof. Secondly it was motivated that the analysis of the four basic diets would be sufficient as a method of maintaining knowledge of chemical compositions of the diet.

The techniques used by UP Nutrilab for the above analysis were as follows:

- DM and Ash: Association of official analytical chemists, inc (AOAC, 1980)
- CP: Association of official analytical chemists, inc (AOAC, 2000)
- EE: Association of official analytical chemists, inc (AOAC, 2000)
- NDF and ADF (Van Soest et al., 1991)
- Starch: Association of official analytical chemists, inc (AOAC, 1984)

5.8) Measurements

The following variables were determined during the study:

Feed intake, per animal/ day (Day 1 - 35)

Green plastic drums were used for the weighing out of feed prior to feeding as well as for weighing back of the orts. Removal of the orts and the weighing thereof was structured in such a way as to limit the amount of time that each steers were without feed to an absolute minimum. Orts removal therefore began at pen 1 and continued through until pen 72. Directly after orts removal, that specific days allocated feed was emptied into its allocated green drum after which it was returned to its allocated pen and the steer was fed. Intake was simply calculated by subtracting the orts from the amount fed daily. The previous day's intake determined the amount fed on any specific day by simply allocating 10% more than the previous day's intake to ensure that no animal was without feed during the trail. If at the afternoon feeding, a an animal seems like it would not have sufficient feed to last for the duration of that evening, extra feed was allocated based on the discretion of the trial coordinator. Prior to weighing back orts or weighing out feed, the feed scale was checked using weights specially designed and purchased for the trial. Those 20kg weights were manufactured and calibrated with the most accurate of scales for the purpose of checking all scales used for feeding, feed mixing and cattle weighing to ensure the utmost could be done to prevent error before as well as during the trial.

Individual live-weight (weekly)

The weekly process of weighing of all animals included day 1 and also the last day of the trial (Day 35). Animals were weighed before feeding on the designated days after which they were returned to their individual pens where fresh feed was available. The scale was calibrated prior to as well as after weighing in exactly the same fashion as the above mentioned feed scale. The weights for day 1 of the trial went amiss and as a result the values attained from weighing on day -3 were used for regression analysis of raw data as well as for statistical analysis.

Faecal score, per animal (Day 1 – 28)

Three Faecal scores per pen from fresh droppings were taken daily from day 1 up until day 28 of the trial. Faecal scoring per pen was done just prior to feeding in the mornings. After scoring, each animal was removed from its single pen for 5-10 minutes giving time for the animal to receive sufficient exercise as well as for the pen to be cleaned thoroughly making the following days scoring as efficient and accurate as possible while providing clean pens for the comfort of each animal. The faecal scoring system is based on a points scale from 1- 5 (Hall, 1999).

Point scale:

1 = dry heaped manure with clear concentric rings, typical of animals eating roughage

2 = manure less dry staring to lose the concentric rings

3 = manure forms a consistent heap with little spreading

4 = manure spreads out on the ground, could indicate digestive disturbances

5 = watery manure spreads out on the ground and is absorbed into the ground; blood may be present and indicates serious digestive disturbances.

Faecal samples

A single faecal sample was collected per animal per treatment, 96 hours after the steers had made the transition onto the 100% grower diets for the first time and the samples were analyzed for pH directly after sampling using a portable hand held pH meter (IQ Scientific palm pH/mV/Thermometer). These samples were taken directly from fresh feacal piles and after determining pH, were frozen for future starch analysis (MacRae & Armstrong, 1968). It would have been ideal to take these samples directly from the rectum of each animal in the form of grabs, however due to the impracticality as well as a conscious effort to prevent putting the steers under too much stress, sampling from fresh piles was preferred. The faecal starch percentage was determined by UP Nutrilab, University of Pretoria from dried faecal samples. These dried samples were obtained from the original frozen faecal samples which were thawed and then oven dried for 48 hrs at 60°C.

Animal health

Morbidity (number of pulls and re-pulls) and mortality (detailed necropsy report) was monitored throughout the trial. Animals that showed signs of acidosis, or any other ailment, were immediately examined and diagnosed and treated accordingly. The 4 steers that were suspected of suffering from sub-clinical acidosis (pen 1, 40, 42 and 59) that showed signs of laminitis were treated with 5ml Phenylarthrite and the treatment was

repeated 48 hrs later. The other 4 steers that suffered from diarrhea were treated with 10ml Depocillan and 1ml Kyrovite A until they no longer showed signs of the problem. Non-severe cases therefore received appropriate treatment, whilst severe cases such as in the case of the steer in pen 1 were treated and kept in isolation until further action could be taken and decisions made regarding its returning or removal from the trial entirely. The latter received its experimental diet during recovery and intake was determined until a final decision was made after which it was fed good quality hay ad lib only.

From the above measurements the following was calculated:

- ADG (per week as well as for the total period)
- Net Weight Gain (NWG/total period)
- DMI (kg/ day per week as well as for the total period)
- FCR (kg DMI/ kg weight gain for the total period)
- Energy Conversion Rate (MJ intake/ kg weight gain for the total period)
- Day-to-Day variation in DMI (kg/day)
- Pulls; Fecal scores and fecal pH

5.9) Statistical analysis

The trial data was analyzed using the program, GenStat (2005), and"Animal" was the experimental unit for all variables, unless otherwise stated. Factors included in the study were the different diet regimes as well as inclusion levels of roughage in the diet. The data to be analyzed included individual data (animal weights, individual intake, fecal scoring, fecal pH and "pulls") and group data (intake per treatment, net weight gain, average daily gain, feed conversion rate, energy conversion rate, DMI/kg body weight

and MJ intake/kg body weight). The study was carried out as a regression design making use of two different diet types with each diet having six individual roughage starting levels. The aim of Regression analysis is to determine a function which best fits the values of the given parameters in the trial (Altman, 1991). In most cases in this trial, a linear regression was used to fit the values. A linear regression is the most basic form of regression analysis and the function is a linear equation which is used to explain how the variation in an outcome variable, Y, depends on the variation in a predictor variable, X. However in certain situations where the outcome will depend on more than one explanatory variable such as in cases in this trial, this leads to a multiple regression. In both cases, a line will be plotted onto the given variables (Campbell and Machin, 1993). The amount of variation in the dependant variable that is accounted for by variation in the predictor variables is measured by the value of the coefficient of determination, often called R^2 adjusted (Altman, 1991). The closer this value is to 1 then the more the regression model is accounting for all the variation in the outcome variable. Below are the given equations for both straight and multiple regression lines respectively.

$$Y = b_o + b_1X$$

$$Y = b_o + b_1X + b_2Z + b_3XZ$$

The level $P<0.10$ was used to define statistical significance. A level of $P>0.10$ but <0.15, although not statistically significant, was used to indicate a tendency towards statistical significance.

Chapter 6: Optimizing the adaptation rate of feedlot steers dosed with *Megasphaera elsdenii* and fed high starch diets: (ii) Results and discussion

6.1) Introduction

The purpose of this trial was to determine how quickly feedlot steers originating from the veld with no previous exposure to concentrate feeds could adapt to a typical feedlot finishing diet (top diet) with a roughage content of 5% after being dosed with the DFM, *M. elsdenii* MeCH4. The performance of animals on the six treatments per substrate group, chop and maize, were therefore compared based on general health, intake and body mass data to determine the impact that the above DFM has on the most critical phase of a feedlot period, the adaptation phase (day1-35). Comparisons between the two substrate groups, chop and maize, were also made. The purpose of this chapter is to analyze and discuss the results on DMI, animal weights (measured weekly), ADG, Net weight gain (NWG), FCR, energy conversion rate, DMI/kg BW, MJ Intake/kg BW, fecal scores, fecal pH and "pulls". This will shed more light on the effect that *M. elsdenii* MeCH4 has on this critical stage of the feedlot period. Due to the nature and lack of research carried out prior to the trial on *M. elsdenii*, comparisons with previous data are difficult especially under feedlot conditions as very little data exists. In the discussion, the results of this trial will be compared to trials that were similar in nature in order to establish the effectiveness of *M. elsdenii* in preventing acidosis, increasing productivity as well as reducing the required adaptation period for feedlot steers.

6.2) Experimental diets

As expected there were slight differences in the predicted and actual values obtained from analysis of the four basic trail diets based on nutrient content. The differences between predicted from Table 2 and actual analyzed values is illustrated in Table 3.

Table 3: Nutrient profiles of four basic diets according to chemical analysis

Nutrients (% DM)	Chop Starter	Chop Grower	Maize Starter	Maize Grower
CP	14.1	14.2	14.7	14.5
Starch	22.9	30.9	40.7	51.9
NDF	45.6	36.1	30.2	19.7
ADF	19.1	14.6	16.6	9.87
EE	6.26	6.83	3.40	3.45
Ash	4.89	4.66	4.75	3.74
Moisture	8.85	10.6	9.80	11.4
Lignin	5.22	5.43	5.46	5.23
NFC	29.2	38.2	46.9	58.7

Variation in NDF can be attributed to the database used, the natural variation in NDF that occurs within feedstuffs and the significant effect of growth stage on the NDF content of roughages. The NRC (2001) published NDF values of 21.1% (+- 5.5) and 42.5% (+- 8.4) for hominy chop and wheat bran respectively, compared to South African values of 49.1% for hominy chop and 48.1% for wheat bran (Dugmore, 1998). Published South African values on the NDF content of Eragrostis curvula (%DM) varied from 63.3% (14% CP) (Erasmus et al, 2008) to 74.4% (8.3% CP) (Dugmore, 1998). Compared to the predicted nutrient values for the four basic diets shown in table 2, it is clear that the CP

values of the analyzed data in Table 3 are very similar; the NDF levels for the analyzed values, however were on average 11% higher than those of the predicted values.

6.3) Cell counts

The reported viable cell counts for *Megasphaera elsdenii* on the respective dosing dates are reported in Table 4.

Table 4 Viable cell counts of MeCH4 samples when feedlot steers were dosed

Sampling date and time	Average viable cell counts (cfu/ml)
06/11/2008 (08:00)	1.53×10^9
06/11/2008 (08:05)	1.33×10^9
06/11/2008 (12:00)	2.07×10^9
06/11/2008 (12:05)	1.53×10^9

[1]cfu: colony forming units

The viable cell counts of the samples taken were well within the recommended cfu levels of 1×10^8 cfu/ml.

6.4) Results on dry matter intake

Results of the ANOVA conducted on DMI, DMI Variation and DMI as a percentage of body weight are presented in Table 5. Regression results for DMI, DMI Variation and DMI as a percentage of body weight are presented in tables 8 and 9.

If one takes a closer look at table 5, we can see that throughout the duration of the trial (day 1-35) as well as during the trial, (day 1-7) and (day 1-21), there was no difference in

feed intake between treatments for both chop and maize based diets. There was, however, a tendency towards a difference for treatments in the maize based diets (Day 1-7) *(P* = 0.13). This difference was attributed to the maize treatment taking 9 days to reach top diet (DRTD), which was more a function of a few animals that took longer to adapt to the individual pens rather than an actual function of the diet. Although this difference in DMI is not significant ($P < 0.1$), a *P*-value of between 0.1 and 0.15 is regarded as a tendency towards significance and is worthwhile noting. These results are contrary to results obtained by Choat et al. (2002) as well as Burrin et al. (1998). The study by Choat et al. (2002) in which adapted steers were fed, showed that steers that consumed an 85% concentrate diet from day 1 as opposed to adaptation over a 5 day period from a 70% to a 85% concentrate diet had a DMI 22% lower than the adapted steers. According to Burrin et al. (1988) similar results were found with a subsequently lower DMI for steers started on the top diet (with the highest concentrate level) from day 1. The same applies to DMI variation as well as for DMI as % of BW where no significant differences for the different treatments can be seen. This suggested that one would be able to decrease the roughage levels and therefore increase the starch levels in the diet from the start of the standing period without a significant negative effect on the DMI parameters. If one were to take a look at table 7 which depicts the regression analysis for the animals fed the chop based diet, one would see that there is a significant difference for DMI (Day 1-7), *P*= 0.03. This significant difference can be attributed to the treatment 1 DRTD which had a DMI of 2.14 kg which was significantly lower than the average of 2.56kg over all 6 treatments for that specific feeding period ($P > 0.10$). There was however no significant difference between the various chop treatments over the entire feeding period although a

numeric difference in DMI did exist. In the case of the maize based diets, table 8 shows that there is no significant difference in DMI between the different treatments.

With regards to the variance in DMI between chop and maize diets, steers fed the chop based diets consumed a significantly lower amount of feed ($P < 0.01$) as opposed to steers fed the chop diet in the initial period from Day 1-7. Over the entire feeding period however, no significant difference existed. If we take a closer look at table 5 we see that there is in fact a numeric difference in DMI between the 2 diets with the steers on the chop based diets consuming slightly more than those on the maize based diet (5.23kg Vs 5.07kg).

The values repeated in this trial for DMI over the first 35 days of the standing period for both diets are significantly lower than the average DMI for the local feedlot industry as well as compared to other trials conducted under feedlot situations. According to Choat et al. (2002), he found a DMI of 8.18kg per day for the first 28 days of his feeding trial. Although the steers used in his trial had an average weight of 287 kg at the beginning of the trial which was 60 kg heavier than the steers used in the chop based diets and 56kg heavier than those used in the maize diets, this heavier starting live weight could not have been the only factor resulting in a higher DMI of almost 3 kg more than observed in this trial. Similar results were reported in a study by Meissner at al. (1995) who used Bonsmara steers of very similar starting weights to those in this trial (239 kg). Meissner et al. (1995) observed a DMI of 7.6 kg/day. This DMI was however over a longer feeding period (105 days) and one would have expected that the DMI of the steers in this trial on

both diets would have been slightly higher than those witnessed due to the increasing body weights of the steers as well as them becoming slowly more accustomed to single pen conditions.

A possible explanation for the lower DMI values in this trial could be attributed to two factors. Firstly, although one would expect that the steers should have been adapted, a possible explanation for the lower intakes could be due to the steers not being completely adapted to the single pen environment at the start of the trial. However under normal feedlot conditions, most cattle would not have been back grounded prior to entrance to the feedlot pens and therefore should have experienced similar stress to those in this trail. One could argue though that the single pens prove to be more stressful than expected due to the limited interaction allowed between animals as well as limited movement in the small pen area. The second possibility would be with regards to the maize based diets and had to do with the grinding of the maize. The possibility exists that the maize was ground too fine which would have negatively influenced DMI due to the dustiness of the feed and the reduced palatability.

Although no differences existed between treatments for DMI variation, there was however differences between diets with steers fed maize based diets showing far greater DMI variation than the chop diets (Table 5). Regression analysis of the chop intakes suggested that there were significant differences between treatments for DMI variation from day1-7 and day1-21 (P= 0.05 and P= 0.09 respectively). For both chop and maize based diets, DMI variation appears to be slightly higher than what one would expect

under normal feedlot conditions. According to Schwartzkopf-Genswein et al. (2004) the values from this trial are in line and the slightly higher day-to-day fluctuation in intake in single pens when fed ad lib was to be expected. Schwartzkopf-Genswein et al. (2004) found DMI variation values of 1.64kg for individually fed steers. This is supported by Stock et al. (1995), who stated that DMI variation in trials where steers were individually fed were seen to be up to 5 to 10 times greater than the variation seen under commercial feedlot trials.

There was no significant difference, for diet or treatment, when evaluating DMI as a % of body weight (BW) for the entire feeding period. There was however a significant difference between diets if the first week is excluded (Day 8-35), (P=0.02). In this instance, DMI as a % of BW for the chop diet was significantly higher than that of the maize diets with a value of 2.34 %. The reason for this difference can most probably be attributed to, as stated before, the reduced palatability of the maize diet due to severe grinding effects leading to lower DMI levels. No literature could be found to support this assumption, although it is well known that finely ground wheat is powdery and therefore unpalatable (MacGregor, 200)

6.5) Growth performance parameters

Growth performance parameters are reported in Table 6. The average initial live weight for each treatment was between 223 and 233 kg except for Treatment 9 (Maize, 13 DRTD) which was slightly higher (239kg). The reason for this was due to the fact that the one animal was identified by Genstat as being beyond the scope of the normal

distribution and were therefore not included in the analyses. Removal of this animal from treatment 9 meant that the average weight of that treatment was slightly skewed. It was the intention of the trial that the weights of the animals purchased firstly be as uniform as possible, and secondly that they represent that of typical steers entering a feedlot locally. The purpose of this was to simulate a typical feedlot situation as closely as possible in order to come to the most accurate and useful conclusion. Taking a closer look indicates that there were no differences in average daily gain (ADG) between the different DRTD treatments in each of the Chop and Maize based diets. In fact, the ADG values in Table 6 for both time parameters, (day 1-35 and day 8-35), were very similar for both the chop and maize based diets resulting in very little numerical differences being seen. As a result we find a *P* value of 0.90 and 0.73 for the respective time period parameters. Over the entire feeding period, steers fed the chop based diets had an ADG of 1.54 kg/day. On the other hand, steers fed the maize based diets had a significantly lower ADG of 1.16 kg/day. These ADG values, especially those for chop compare very well to other published research material. In a study reported by Stock et al. (1995) sixty angus steers with an initial BW of 261 kg were used to test the effects of monensin levels on steer performance. These steers were individually fed by making use of Calan Gates and were previously adapted to this feeding system. The steers were fed for a standard feedlot standing period. Stock et al. (1995) found ADG values to be in the region of 1.25 kg/day. When comparing these values to those for chop and maize diets in this trial, we see that steers consuming the chop diet grew on average 23 % more per day than those in the opposing trial. However when comparing the values of the maize based diet we a 7 % lower ADG is achieved. In another study conducted by Meissner et al. (1995), values for

ADG of 1.56 kg/day were obtained for Bonsmara weenlings of similar starting weights to those of the steers used in this trial. Although these values are far higher than the ADG values for steers on the maize based diets in this trial, they are indeed very similar to those values for the chop diets. One would tend to think that the steers in this trial out performed the steers in the trial conducted by Meissner et al. (1995), as they stood far longer (105 days) and the steers were also not subjected to the same stresses as associated with single pen trials. Lastly, in a trial conducted by Fluharty and Loerch. (1996), ADG for the 28 day feeding period was 1.05 kg/day and 1.11 kg/day for steers fed a 70 % and 85 % concentrate diet respectively. In their trial seventy seven Simmental crossbred steers were used with an initial BW of 226 kg. Steers fed both the maize and chop based diets out performed steers from the above trial, with those fed the chop based diet performing 41 % better with respects to ADG.

From this data we were able to conclude that even though the DMI values for this trial which we conducted were slightly lower than those seen in other trials as well as in the feedlot industry, the same reduction in performance with regards to ADG was not seen. One question does however arise with regards to why the ADG of steers on the chop treatments were so much higher than those on the maize treatments? A significant difference ($P < 0.01$) between the two diets for both time periods (Day 1-35 and Day 8-35) was noticed. The answer to this question seems relatively simple when one considers that the DMI for the chop based diets was significantly higher for the feeding period especially from day 8-35 as steers based on the maize diets had higher DMI in the first week as opposed to those on the chop diet. Although the energy value for whole maize is

higher than that of hominy chop, it would seem that this difference in energy alone was not enough to buffer the difference in DMI resulting in a drop in performance with regards to ADG.

The reason for the incorporation of the parameter (Day 8-35), was to identify the effects of steers becoming fully adapted to the single pen environment. If one takes a look at ADG for the two periods we find a significant difference between the two periods. The ADG value for Day 1-35 was 1.54 kg/day and 1.16 kg/day for chop and maize respectively which increased to 1.77 kg/day and 1.27 kg/day respectively for Day 8-35. This difference can be attributed not only to the unfamiliar environment and size of the pen but also to the unfamiliar feed as these cattle were brought into the trial straight off the veld.

Feed conversion ratio (FCR) analyzed by using the ANOVA method, is reported in Table 6. There was a significant difference (P=0.08) between the treatments (Day to reach Top Diet (DRTD)) for FCR (Day 8-35). This significant difference can be attributed to a high FCR for maize the treatment, 9 DRTD, as well as significantly poorer FCR for the maize treatment, 17 DRTD. Although for the parameter, FCR (Day 1-35) there was no significant difference, there was however a noticeable difference and a definite numeric difference (P=0.16). When compared to data from other trials it seems that the FCR values compare rather admirably. In the trial by Meissner et al. (1995), they found FCR values between 3.79 and 4.10 kg DMI/ kg BW gained. When a closer look was taken at the values of this trial, FCR values for chop based diets average 3.55 across all

treatments. In comparison, the maize based treatments averaged 4.53 kg DMI/ kg BW. Although the values for maize are slightly poorer FCR, those for the chop diet are lower (better) than the comparative values from Meissner et al. (1995). In another trial by Stock et al. (1995), FCR values of between 5.37 and 5.66 were given. The FCR values for the maize based treatments in our trial were very much in line with these values as can be seen in Table 6. The chop FCR values are once again however significantly lower. This then begs the question of how the two diets compared against each other. Table 6 indicates that as suspected, there is difference ($P<0.01$) between the two diets. This situation is presented for both parameters from (Day 1-35) as well as from (Day 8-35). As a result we have seen that the chop based diet far out performed the maize diet and required on average 23 % less feed per kg to produce 1 kg of body mass. The next question that springs to mind is therefore what caused the enhanced performance of feedlot steers fed chop based diets as opposed to those fed maize based diets? This proves a very difficult to answer as there seems no conclusive prior research to prove this point. To venture a reason is risky but one possible explanation for this could have to do with the higher NDF fraction in chop as opposed to maize. The NRC Dairy (2001) reported average NDF values of 21.1 and 9.5% for hominy chop and maize respectively while Dugmore (1998) reported a NDF value of up to 49% for South African chop. MacGregor (2000) reported an NDF value of 55% for hominy chop feed, illustrating the wide variation between the compositions and manufacturing process of hominy products. Although this higher NDF fraction would subsequently mean a lower ME value, it would lend to more efficient utilization of the nutrient fraction in the maize. This would be as a result of a slower passage rate through the digestive tract, ultimately leading to a higher

mean retention time (MRT) of feed in the digestive tract and therefore more efficient digestion. This allows the microbes residing in the tract more time to effectively ferment feed and produce microbial protein which can later be used by the steer with the end result being I better FCR.

If we take a look at the regression analysis for chop and maize diets in Table 7 and 8 respectively, there was no significant difference between either of the two diets indicating that apart from slight numeric differences, the treatments within each of the diets performed relatively similarly throughout the duration of the trial.

Results on fecal parameters, namely fecal score, pH and starch level as well as the number of pulls, analyzed by using the ANOVA method are shown in Table 6. Fecal scores for both diets increased significantly (P=0.08) as the DRTD decreased. The regression analysis of the data indicate tendencies towards significant differences for both diets however there is no differences for fecal scoring. The increase in fecal scores as DRTD decrease were expected. This would be due to the fact that as the amount of concentrate in a ration increases the looseness of the feces then increases due to the significant reduction of fiber in the ration which is more associated with the roughage component of the diet. The average scores obtained in this trial are still well within the range, if not slightly lower, of those seen in the local and world wide feedlot industry.
The average fecal score for the chop diet was 2.10 which was lower (P<0.01) than that of the maize diet fecal score of 2.25. The reason for this difference would obviously be due

to the much higher starch levels in the maize and the resultant lower NDF fraction of the maize based diets.

Fecal sampling was carried out 72 hours after the steers started to consume the Top Diet. From Table 6 we saw a significant reduction ($P<0.01$) in fecal pH as the DRTD increased. The results of the individual diets are as follows, the average fecal pH for chop based diets being 6.97 and for that of the maize based diets was 6.15. These results compare differently with other previous studies but seem to be consistently lower however. When compared to results from Fulton et al. (1979), the pH values found in this trial appeared far lower. The trial by Fulton et al. (1979), involved steers adapted to high concentrate diets based either on corn or wheat as the major starch contributors. In this trial, steers fed the maize based diet had an average rumen pH of 5.59 on the 3rd day of the trial. Although in this trial a fecal sample was taken to depict the happenings in the rumen, it is widely appreciated that a fresh fecal sample is very representative of actual pH values in the rumen and lower digestive tract. With respect to the wheat based diets, a ruminal pH of 5.54 was obtained on the 3rd day after onset of feeding. These values as can be seen are much lower to those in our trial. This can most probably be attributed to the significantly higher levels of *M. elsdenii* in our trial due to the initial dosing prior to the onset of feeding. These levels of *M. elsdenii* therefore probably reduced the levels of lactic acid produced in the rumen which would under normal circumstances be the cause o such a sever reduction in pH. In a study by Bevans et al. (2005), pH values of between 5.62 and 5.70 were noticed. These values are slightly higher than the values from the trial by Fulton et al. (1979) but relatively similar but once again much lower than the values

from our trial. The reason for this would presumably be the same as for the previous trial. In the trial by Bevans et al. (2005), ruminal pH values were even lower for those animals which were transitioned on to the so called top diet immediately. This is however not the case in this trial.

In a trial conducted by Brown et al. (2000), 5 steers were challenged with a concentrate diet after being forage fed or fasted for a significant period. In this trial, 3 out of the 5 steers had a rumen pH of below 5.0 which resulted in nearly total avoidance of feed a day after the onset of feeding. Only 1 of the above steers had no significant effects regarding symptoms concerning rumen acidosis. It could therefore be speculated that without dosage of *M. elsdenii* to combat the negative effect of lactic acid producing bacteria, the pH of the steers in our trial would probably be significantly lower and in some cases might have resulted in cases liked with symptoms of either acute or sub-acute rumen acidosis.

With regards to the pH values from our trial, one would expect that an increase in the starch levels in the diet would result in a decrease in the fecal pH. Under most circumstances, this would be the case. However in our trial this was found not to be the case. An explanation for this was the fact that fecal samples were not collected from each group of steers on the same day. Instead samples were collected on different days based on when the group was transitioned onto the top diet. Because of this the steers that took longer to reach the top diet (Higher DRTD) had lower fecal pH values relative to those sampled earlier. The cause of this being the fact that by the time the later groups were sampled, their rumens had sufficient time to adapt to the concentrate diet and handle the

lactic acid load produced and therefore lessen the negative impact it would have had if allowed to accumulate.

Closer inspection of Table 6 indicates also a significant difference ($P<0.01$) between the two diets. The steers on the maize based diets had a lower fecal pH than those on the chop based diets. Steers on the chop diets had an average fecal pH of 6.97 while those on the maize diet had an average value of 6.15 ($P<0.01$).

The fecal samples were also analyzed later for starch content. There was a significant difference ($P<0.01$) in fecal starch % between the two diets. Whereas the steers on the maize based diet had an average fecal starch % of 11.94, the steers on the chop diet had an average fecal starch % of 2.29. The answer as to why the values differ so greatly seems to be relatively simple. Firstly as analyzed, the hominy chop used in this trial had afar lower starch content than that of the maize. Secondly due to the fineness of the chop, the starch is far more accessible to rumen microbes for fermentation. This along with the longer retention time in the rumen discussed earlier probably would have lead to far higher levels of starch fermentation and therefore higher levels of utilization than compared with crushed maize. One must ask in that case why then does the feedlot industry world wide rely so heavily on maize as the major starch component of the diet if there seems such a suitable replacement in the form of hominy chop? Upon consultation with a local feedlot employee, the answer seemed to be rather simple. The answer lies in the security of supply as well as the reliable quality of hominy chop (R.Watson, Personal communication). Starch content of chop varies greatly dependent on the miller it is

supplied by. This along with the fact that the starch is so much more readily available for rumen fermentation than crushed maize, it means that the possibility of digestive disturbances in steers consuming such a diet, especially those newly introduced to the feedlot situation with no prior adaptation to concentrates, is so much greater. The milling process is also of importance as maize ground too fine as opposed to crushed maize will also likely lead to far more digestive disturbances. This is therefore one reason why *M. elsdenii*, as a dosed pro-biotic, provides a distinct advantage to the feedlot industry. The variability in starch levels in hominy chop is not however the only problem. Chop also has a higher fat content than maize. Although this means a slightly higher energy content than maize, it also means that it can only be stored for a short period of time before being fed to prevent the feed from becoming rancid due to the elevated oil levels. This poses other constraints on feedlot managers regarding transport etc.

Taking a look at a study carried out by Depenbusch et al. (2008), the study revealed that there was a negative correlation between fecal pH and fecal starch concentration which is in agreement with the results from our trial. Our study did indeed prove that a negative correlation existed between fecal pH and starch % and that in the case of both diets, as the fecal pH decreased the starch % increased. Between diets the same relationship was noticed and even though steers that were fed the maize based treatment had a higher fecal starch %, they indeed had a far lower fecal pH.

With regards to the between treatment variation form this trial, once again a significant difference (P=0.04) was found. It seems as if the starch content decreased as the DRTD

decreased. As mentioned earlier, the fecal samples were collected on different days for the different treatments which although was not ideal and ideally we would have liked to sample all animals on the same day, this was not possible due to the fact that the treatments reached the same diet composition on different days. It seems possible that this could have had an effect on the starch % which as stated earlier was not desirable as all other variables other than diet would have wanted to be eliminated. It seems that the longer the animals consume the feedlot diet, the more starch seems to flow through to the feces. This could be explained by increased levels of intake as they adapt to the diet. Therefore as intake increases, starch digestion may decrease due to a change in MRT resulting in higher concentrations of starch in the feces. This could possibly however puts the reliability of fecal starch as a viable measurable parameter into question? But due to lack of substantial, conclusive previous research this is speculative more than fact.

Pulls in all treatments of both diets were very low as can be seen in Table 6. According to Brown et al. (2006), steers with no previous adaptation to concentrate diets which then consume feed based on starch concentrates such as maize or chop would in almost all instances show symptoms of both acute and sub-acute rumen acidosis which if left untreated would result in decreased performance or even death. One would therefore expect that as the DRTD in our trial were to decrease (higher concentrate diet being fed) then the number of pulls would most probably have increased. This is however not the case and as we can see from Table 6, there was not one single pull from either of the two top treatments for either the maize or the chop diets. This means that the steers from the two treatments consuming the highest concentrate levels in the diet from day 1 showed

no adverse effects what so ever, not even other symptoms believed to be associated with sub-clinical acidosis such as laminitis which in our trial was the major contributor to reasons for steers being pulled from their feedlot rations. Our results therefore suggests that one could reduce the DRTD or totally do away with adaptation phases without any negatively associated health issues which once again promotes the use of *M. elsdenii* as a dosed pro-biotic. One should however bear in mind that the study time for our trial was only 35 days.

Metabolizable energy (ME) intakes of steers consuming the different treatments are reported in table 9. There was no difference in ME intakes between treatments in either of the maize or chop diets although a numeric difference was seen and ranged from 56.88 to 64.00 MJ/day (day1-35). This difference can be noted especially in steers fed the maize based diets, where in general the ME intake increased as the DRTD decreased. There was also no difference between diets over the entire trial period. This is due to the lower ME values of the chop diet. Therefore even though over the entire period, intakes by steers fed the maize based diets were significantly lower, there was no significant difference in ME intake due to the difference in ME levels in the two diets.

The picture is however slightly different when we consider the first week of the trial as well as the period Day 1-21. In both cases there was a higher ME intake in the maize based treatments ($P<0.01$) as opposed to the chop treatments ($P=0.08$) for the periods 1-7d and 1-21d respectively. This dietary difference in ME intake could be explained by the fact that over both these two initial periods, DMI was slightly higher in the maize

treatments. This coupled with higher ME values of these treatments resulted in higher ME intakes over the chop treatments. Total roughage intake over the 35 day trial period decreased significantly per animal ($P<0.01$) as the DRTD decreased. This is clearly illustrated in addendum B as well as Table 7 where it can be seen that the roughage level in the diet decreased as the DRTD decreased.

As stated previously, all data was also analyzed using the regression analyses approach (Table 7 and 8). Much of the data represented in these tables tell a similar story to that of the data in the ANOVA tables. Results from the chop based diets are reported in Table 7. There was a significant difference in feed intake for the parameter, Day1-7, as the DRTD decreased ($P=0.03$). The reasons for this reduction in DMI could be as follows. Firstly one could argue that as the starch density of the diet increases, the DMI would decrease as seems the norm in the feedlot industry, due to metabolic effects in the form of acidosis. However from the previous ANOVA tables it seems unlikely that this be the cause of the drop in DMI. All previous data reflects that a decrease in DRTD had no significant effect on conditions of acidosis but that significant differences arose due to other aspects. One of these in this case reverts back to recognition of feed. This seems a more reasonable argument than the former. One could then argue that steers from all treatments would reject their feed as for all treatments having such high concentrate levels. However closer inspection of the actual feed reveals that the treatments with higher DRTD consumed feed that had visibly a far greater proportion of roughage. This proportion obviously regressed as the DRTD decreased. This easier recognition of feed containing visibly more roughage (steers straight off the veld) therefore would initially theoretically be more

likely to consume a greater amount of the feed placed before them. However the longer these steers consume this new feed, the more accustomed they become and the smaller the difference in DMI becomes as is indicated by the lack of a significant difference for DMI of the chop treatments from Day 1-35.

As can be seen from Table 8 and the DMI data for steers consuming the maize based treatments indicates that there was no significant difference between treatments for any of the time periods (P>0.01). This supports the above argument that the differences in DMI was not due to metabolic disturbances as if this was the case the effect would be exaggerated in the maize diets due to the significantly higher starch content.

With regards to DMI variation in the chop treatments, an interesting observation was noted. For the parameters Day 1-21 and Day 1-35, The DMI variation decreased as the DRTD decreased but only as far as the treatment, 13 DRTD. The DMI variation thereafter increased for the remainder of the treatments. It is nearly impossible to state whether or not this is simply coincidence or whether there is a nutritional reason for this pattern but due to its regularity it was thought worth noting as this may indicate a possible breaking point for the optimal time period for adaptation of steers off the veld to high starch diets common to feedlot rations. No other significant differences for chop based treatments were noted for the remainder of the measured parameters i.e that were not intake related.

With regard to the maize treatments, a significant increase (*P*= 0.09 and *P*= 0.04 respectively) in ADG with a decrease in DRTD for both parameters, Day 1-35 and Day 8-35, was observed. Fecal scores also increased (*P*= 0.04) as the DRTD decreased. However as stated previously when ANOVA results were discussed, this is to be expected as an increase in starch level would naturally result in looser feces. But once again these fecal scores are no lower than what is to be expected in the local and international feedlot industry. The fecal pH's decreased and the fecal starch % increased significantly (P= 0.07 and P=0.05 respectively) as the DRTD decreased and again the same reasons as above apply. A change in DRTD did not affect (*P*>0.01) any of the other parameters shown in Table 8. Relevant graphs that depict certain situations more clearly or show significant differences between treatment and or diets are shown in addendum A. The feeding program for the trial which represented the basis of the combination diets is given in Addendum B.

Chapter 7: Optimizing the adaptation rate of feedlot steers dosed with *Megasphaera elsdenii* and fed high starch diets: (iii) Conclusion

There may be one major conclusion to be drawn from this trail and this was the fact that a decrease in adaptation days to reach the high concentrate grower diet had no statistically significant influence on key performance parameters relevant to the feedlot industry. The implications thereof seem very promising. This is contrary to the majority of previous studies which clearly reflected the fact that steers which make the transition from roughage based diets to concentrate based diets without any previous exposure to concentrate type feed would be metabolically challenged. According to such previous research, a metabolic challenge of such a nature would invariably result in manifestations of conditions associated with acute and sub-acute rumen acidosis. It is generally agreed that if these animals were to be left untreated or left to continue consuming the challenging diet, that aside from lower growth performance, the result would most definitely lead to mortalities. It is therefore a given in the feedlot industry that cattle entering the feedlot, either be back grounded on the veld or that a feed supplement be incorporated into the diet which helps lower the risk of acidosis by changing rumen fermentation patterns or by buffering the drop in pH throughout the digestive tract. Even with the aid of such management techniques, animals can't simply be switched from an all roughage diet to a high concentrate grower/finisher diet. This requires a series of adaptation diets (step-up diets) to be incorporated during the initial feeding phase to allow time for the cattle to slowly adapt their digestive tracts and establish sufficient numbers of lactic acid utilizing bacteria such as *M. elsdenii* to decrease the acid load and

help prevent the detrimental fall in rumen pH. This adaptation period can last for a period of anywhere from 10-21 days with up to 2-3 intermediate diets of varying roughage inclusions. Not only is this a time consuming exercise, but it is costly too as from a nutritional point of view, roughage is by far the most expensive raw material in a feedlot diet. Therefore a feed DFM such as *M. elsdenii* which could be dosed prior to the onset of feeding and reduces or eliminates the need for intermediate, higher roughage adaptation diets could have major implications for the feedlot industry.

Most of these implications relates to the production cycle, namely a potential decrease in standing days, increased growth rate and performance and lower digestive related mortality/morbidity rates. A decrease in standing days has large cost saving implications and would be due to the fact that less time would be spent initially on adapting the cattle to the concentrate diet. This could of course result in higher growth rates early on as cattle would be consuming higher starch and energy levels even though they may not be consuming significantly greater amounts of DMI as was illustrated in this study. Lower mortality and morbidity levels are also very important. Although mortality losses are easy to quantify, the amount of production lost due to morbidity in the form of digestive upsets and other acidosis related behavior are much more difficult to quantify. It is felt though that these losses outweigh those related to mortality as it takes a substantial period for affected animals to recuperate from such disorders before once again reaching previous levels of production in the form of DMI, ADG and FCR etc. Reducing the extent of such losses would also contribute to substantial savings in the feedlot.

However, possibly the most attractive implication of these results is the lower need for roughage and therefore the implications tied in with the buying in and storage thereof. It must however be understood that roughage as a component of feedlot diets can't be eliminated altogether. Many nutritionists believe a 5% inclusion to be the absolute minimum inclusion due to the function of roughage in the rumen and its subsequent effects on passage rate and health of the digestive tract lining as well as the survivability of crucial rumen microbe populations. In typical adaptation diets, the roughage level could be around 20% which means that a substantially greater amount of roughage is required for these initial adaptation diets. Most feedlots maintain a 12 month roughage feedbank in case of drought which would limit roughage availability. This means that an incredible amount of storage space is required to keep this supply and costs of transport makes this raw material a serious burden financially, logistically as well as being a risk in the form of a potential fire hazard. Furthermore, roughage is a raw material which contributes by far less nutritionally in the form of energy and starch to feedlot animals than other dietary components.

Results from this study has shown that dosing cattle with Megasphaera elsdenii NCIMB 41125 could have major implications for the feed industry through a reduced adaptation phase and reduced roughage needs.

Addendum A:

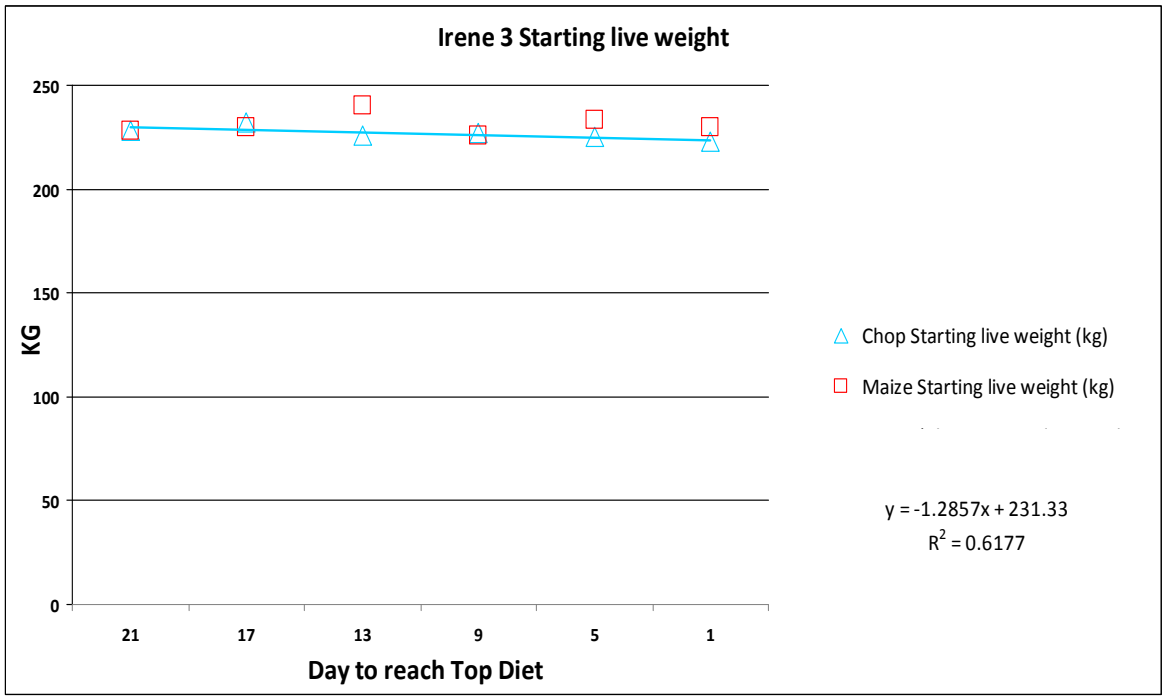

Figure 2 Starting live weights

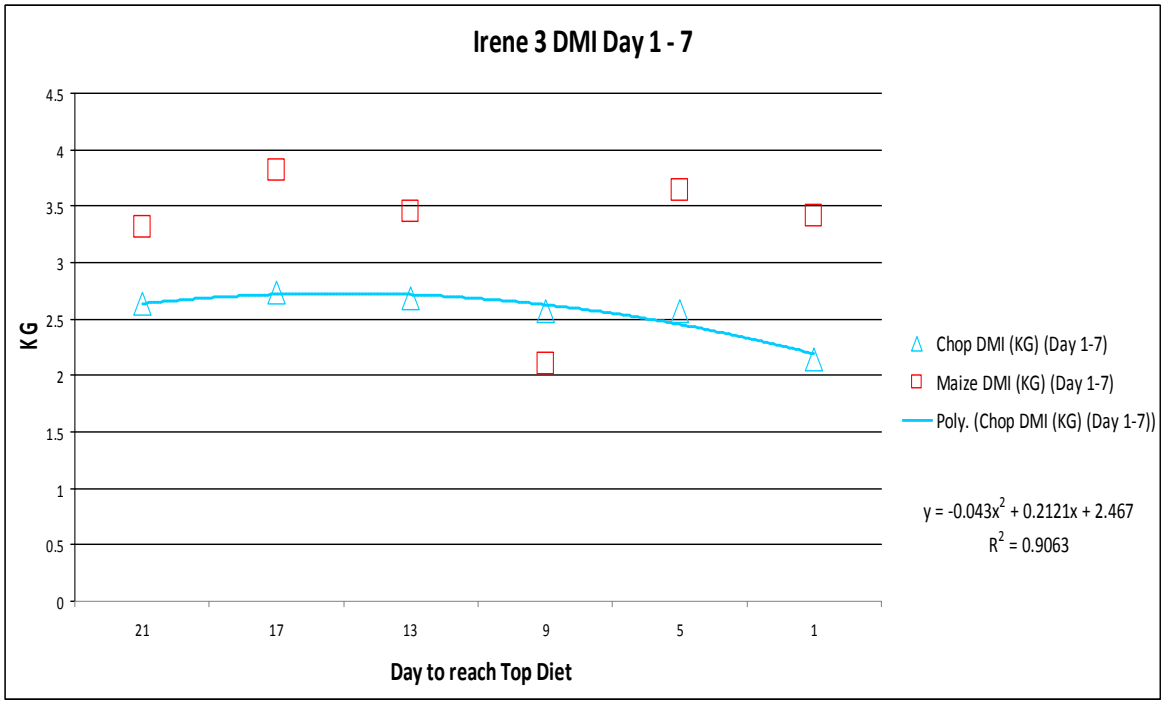

Figure 3 Dry Matter Intake Day 1-7

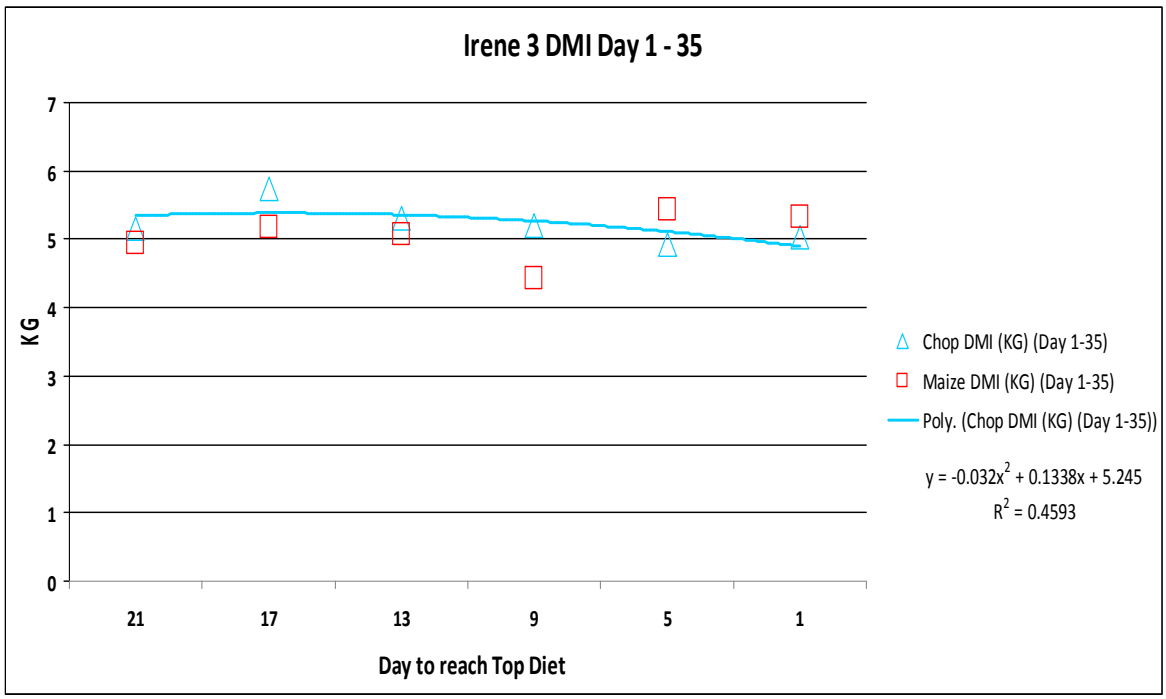

Figure 4 Dry Matter Intake Day 1-35

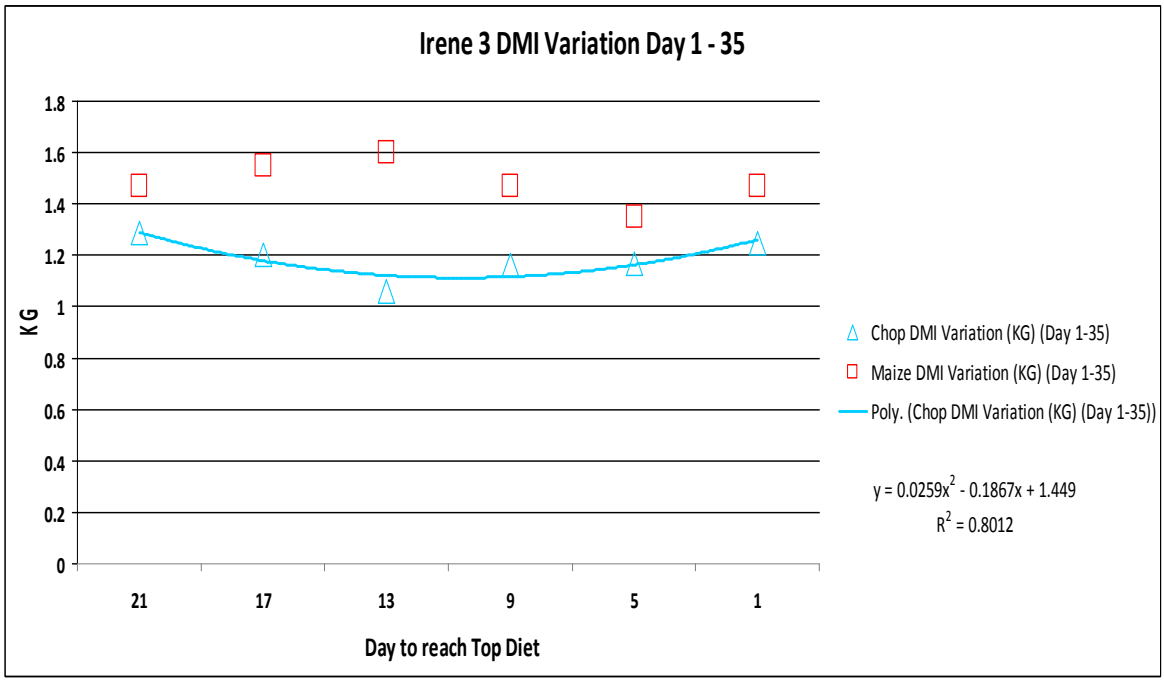

Figure 5 Dry Matter Intake Variation Day 1-35

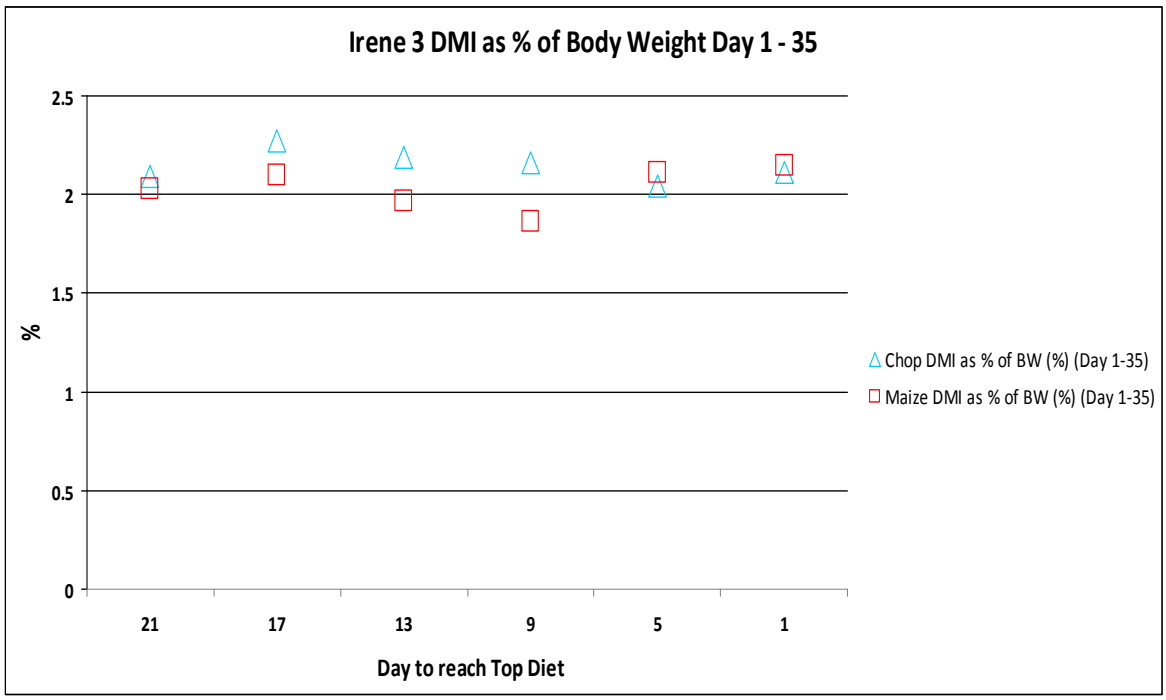

Figure 6 Dry Matter Intake as a % of Body weight Day 1-35

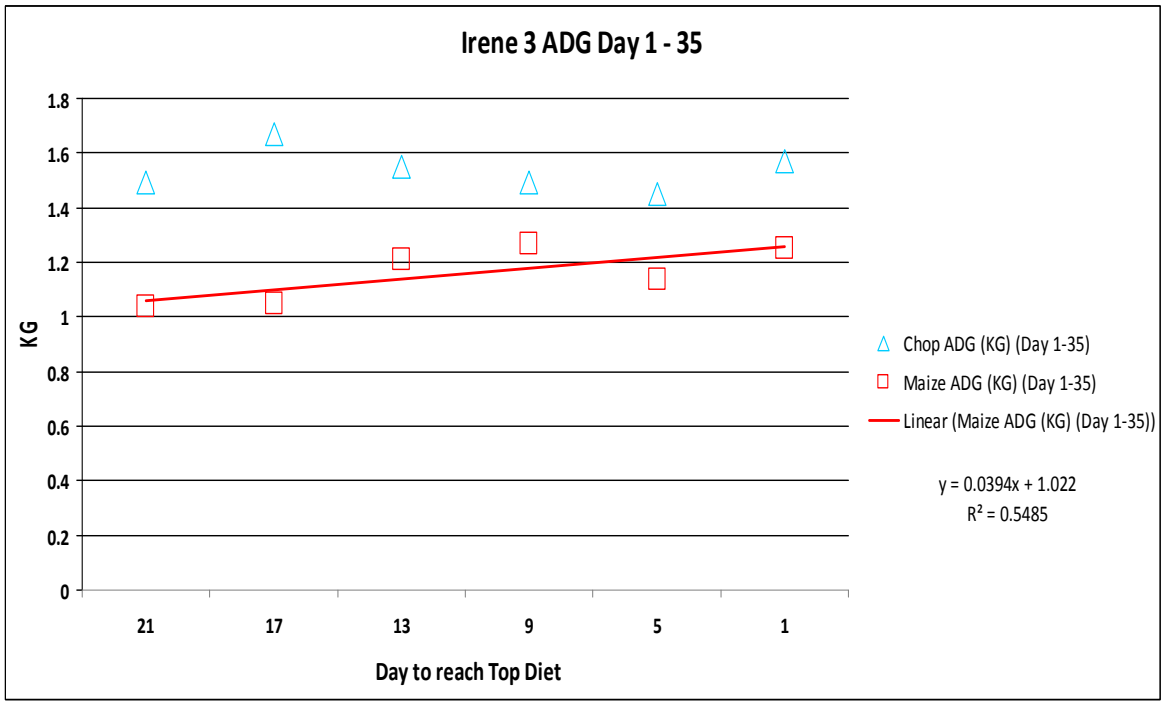

Figure 7 Average Daily Gain Day 1-35

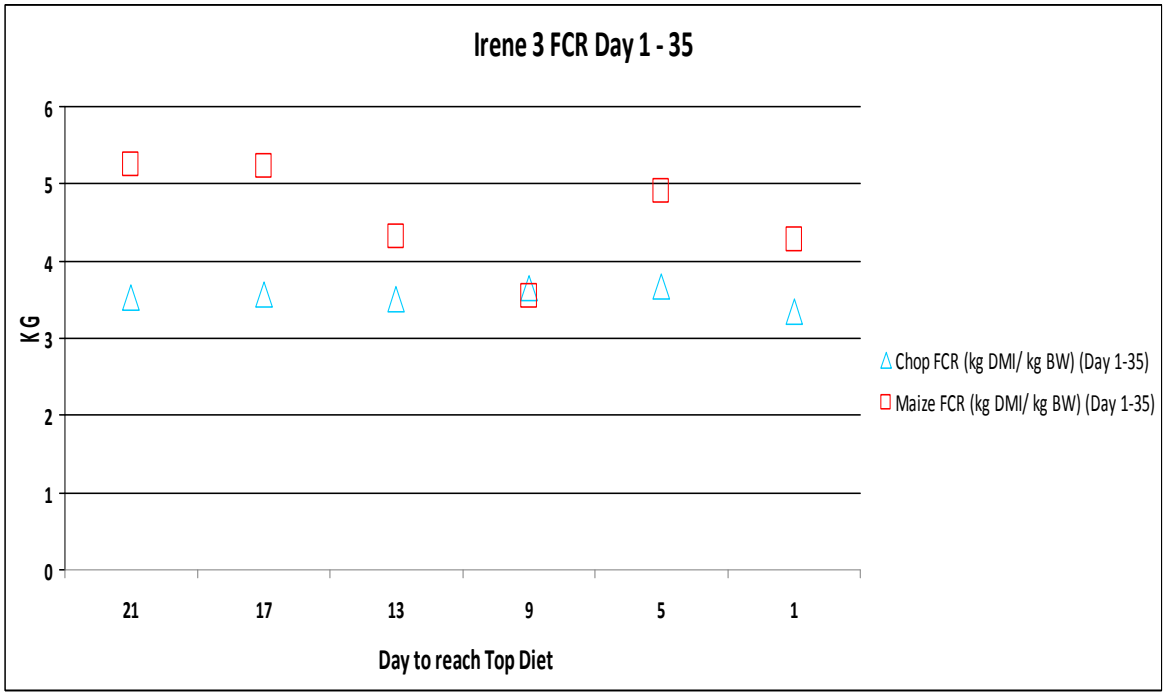

Figure 8 Feed Conversion Rate Day 1-35

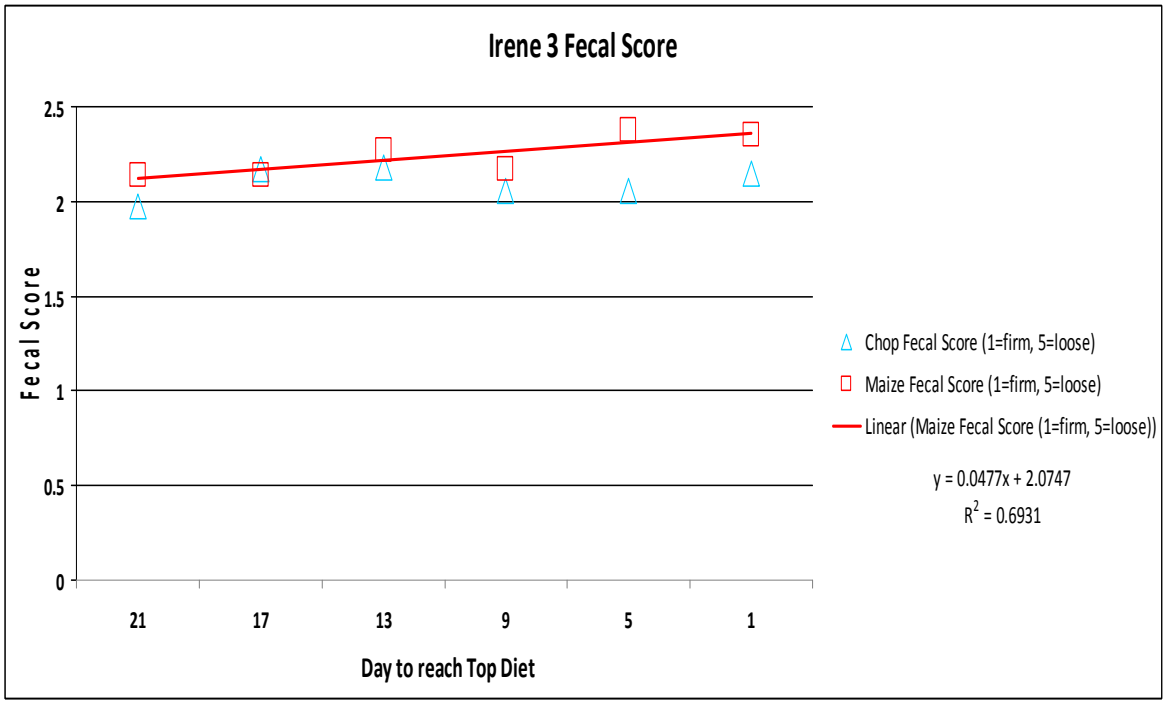

Figure 9 Fecal scores

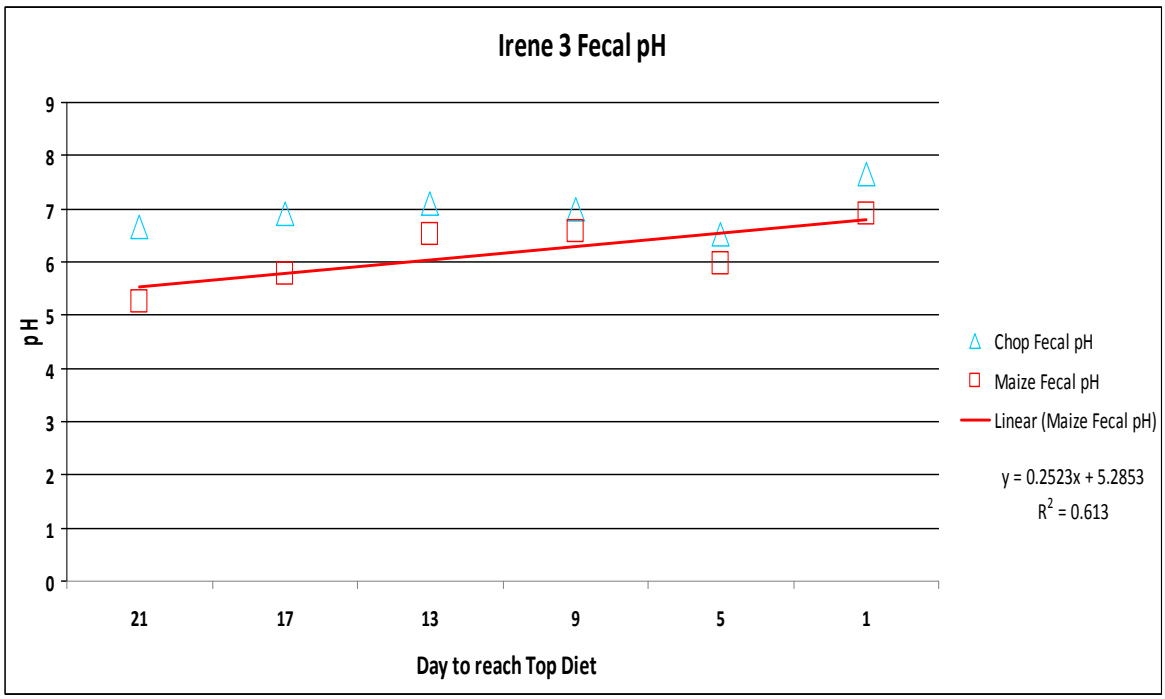

Figure 10 Fecal pH

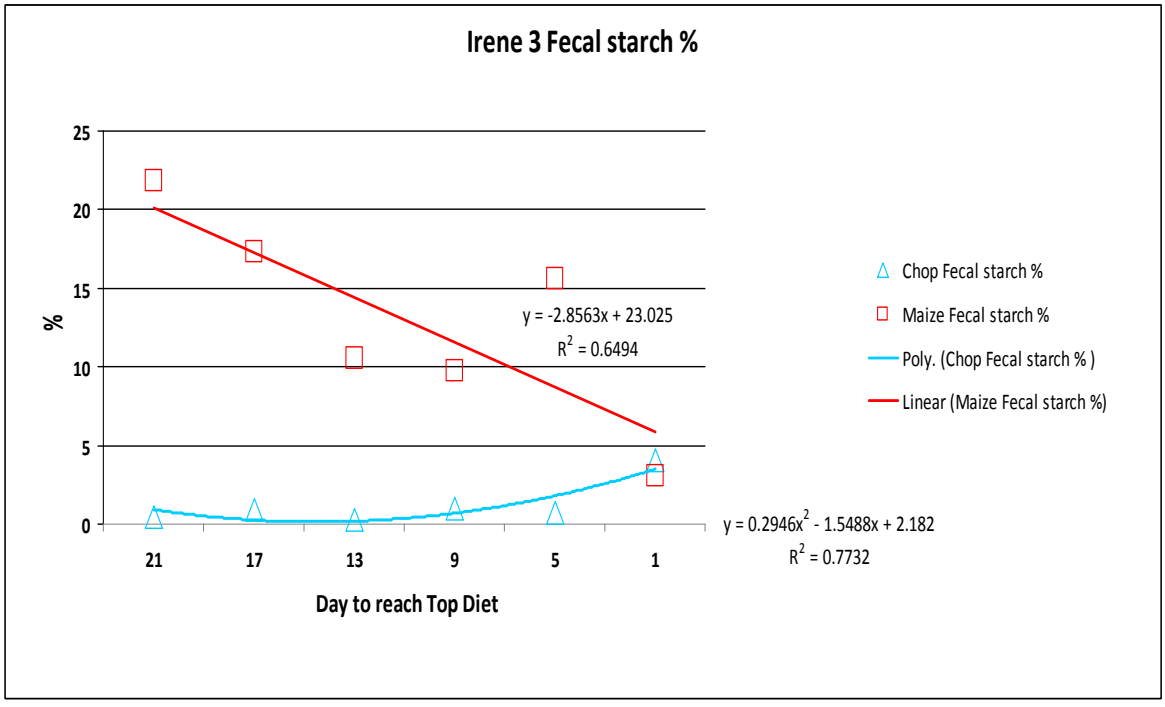

Figure 11 Fecal Starch percentage

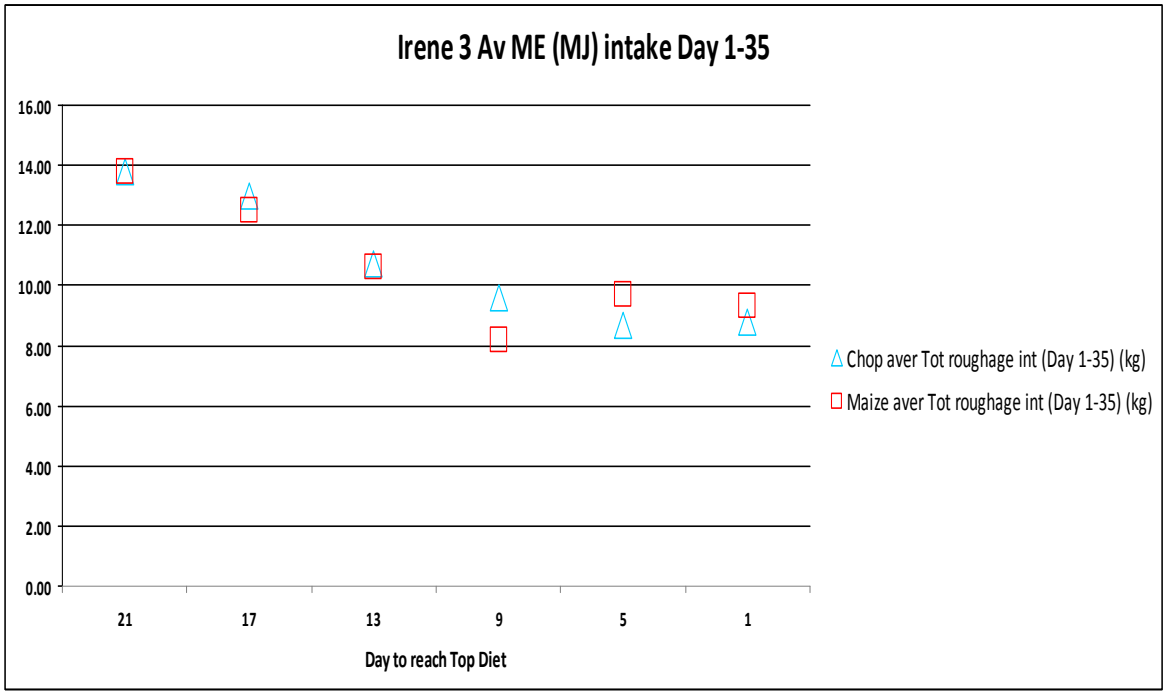

Figure 12 Average Metabolizable Energy intake Day 1-35

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