The effect of *Megasphaera elsdenii*, a probiotic, on the productivity and health of Holstein cows

by

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Contents

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Abstract

Rumen acidosis is a metabolic disorder of ruminants, characterized by a severe drop in rumen pH. This is due to an accumulation of acids, especially lactic acid in the rumen. Lactic acid is one of the strongest acids with a major effect on rumen pH. A low and / or fluctuating rumen pH can have a severe impact on the productivity and health of dairy cattle, especially during the early lactation period. Rumen acidosis can, for example, occur during the rapid change from a low concentrate to a high concentrate diet. *Megasphaera elsdenii* (*Me*) is a lactate-utilizing micro-organism that converts the lactic acid that is produced from the fermentation of starch in the rumen, to propionic acid. Based on the ability of *Me* to convert lactic acid to propionic acid, a study was conducted to determine the effect of dosing live sources of *Me* on the level of rumen acidosis, general health and productivity.

Sixty high-producing multiparous dairy cows were used in a randomized complete block design experiment. Cows were blocked according to milk production during the previous lactation and, thereafter, randomly allocated, within each block, to one of the following treatments:

1) 60% concentrate TMR;
2) 60% concentrate TMR with *Me* dosing;
3) 70% concentrate TMR;
4) 70% concentrate TMR with *Me* dosing.

The experimental period was 60 days and cows were dosed on day 2, 10 and 20 post-partum. Cows were housed in a semi-intensive housing unit equipped with Calan gates for determining individual feed intake. Daily milk production and dry matter intake were measured, as well as body mass and body condition score. Milk was analyzed for fat, protein, lactose and MUN; rumen fluid for pH, volatile fatty acids and lactic acid; faecal samples for pH and starch and feed refusal samples for nutrient components. In addition the health statuses of the cows were also monitored.

In general the results did not show a clear advantage of dosing *Me*, regardless of the level of concentrate. Dry matter intake, milk production, milk composition, feed efficiency, body mass and body condition score were not affected by treatment ($P > 0.05$). Contrary to expectation, treatment did not affect rumen pH, rumen lactic acid or volatile fatty acid concentrations ($P > 0.05$). Faecal pH, however, was higher and the starch content lower in cows dosed with *Me* ($P < 0.05$) suggesting a positive effect on rumen fermentation and more efficient total tract starch fermentation. Furthermore only two cows were culled from the dosed group, compare to eleven from the control group, suggesting a positive influence of *Me* on the general health of stress, early lactation cows. Further research is needed to better quantify the potential role of *Me* in preventing SARA.

Key words: Rumen acidosis; *Megasphaera elsdenii*; Dairy cows; Rumen fermentation; Milk yield; Health

Chapter 1

Introduction

A large number of cattle in South Africa may be classified as high-producing animals. The concept of rumen acidosis is not new for most individuals involved with these intensively fed ruminants. Ruminal acidosis can be defined as an array of biochemical and physiological stresses caused by rapid production and absorption of ruminal organic acids (Krehbiel *et al*., 1995).

Modern day intensive production systems, especially with high producing dairy cows, involve feeding high levels of concentrates in order to supply sufficient nutrients to support high milk production. Feeding these high levels of concentrate can often lead to metabolic dysfunction leading to for example rumen acidosis, if the feeding management is not of the highest level. With feedlot cattle, the aim is to change from roughage-only diets to high concentrate diets as rapidly as possible in order to obtain the highest average daily gain (ADG) and best feed conversion ratio (FCR). The aim of using high concentrate feeding programs is to maximize performance and efficiency, while keeping digestive disturbances such as acidosis within acceptable limits through good nutritional management (Henning, 2004). If acidosis is not controlled, it will lead to problems such as irregular feed intake, reduced milk production, digestive disturbances, possible reproduction and health problems such as laminitis, lung diseases and endometritis (Hall, 1999). Numerous research trials have been conducted worldwide to investigate the occurrence, cause and nature of rumen acidosis, and the effect of rumen pH and high lactic acid concentrations on ruminants.

Theoretically, a number of approaches can be followed to control the incidence of acidosis. One approach is to inhibit the growth of lactic acid producing bacteria such as *Streptococcus bovis* and *Lactobacillus* species through the use of feed supplements such as ionophores. Another approach is to regulate lactic acid levels in the rumen through increasing the population of lactic acid utilising bacteria such as *Megasphaera elsdenii* (*Me*). The latter approach is regarded as a more “natural“ intervention compared to ionophore supplementation and is far more acceptable to today’s consumer of meat and milk products.

Ionophores have been banned in the EU and there is an increasing pressure on producers to find alternatives for antibiotic growth promoters, especially if producers are investing in the export market.

The purpose of the study described here is to determine the effect of the dosage of live *Me*, a natural lactate utilising bacteria, on the productivity and health of early lactation Holstein cows.

Chapter 2

Literature Review

Ruminal fermentation

The rumen is a very complex organ in the ruminant. There are a number of different micro-organisms in the rumen, with the main three groups being bacteria, protozoa and fungi. These organisms are, to a large extent, responsible for the fermentation process of the different feedstuffs to convert the non-usable feed particles to absorbable nutrients, for example volatile fatty acids (VFA). The pH in the rumen also plays an important role in the function of the rumen and its micro-organism population. This chapter provides a brief overview on certain aspects of rumen fermentation in order to provide a background for chapter 3 where rumen acidosis is discussed in detail.

2.1 Rumen bacteria

The rumen contains different groupings of bacteria, the main groupings being proteolytic, cellulolytic and amylolytic. In the context of energy metabolism, the two most important bacterial groups are cellulolytic and amylolytic bacteria, with the latter most significance in respect of high energy diets (Ørskov *et al.*, 1990).

Amylolytic bacteria produce amylolytic enzymes, which are responsible for the hydrolyzation of starch. The number of amylolytic bacteria in the rumen depends on the diet that is consumed by the ruminant. The more starch in the diet, the more amylolytic bacteria (Ørskov *et al.*, 1990). The growth rate of amylolytic bacteria may affect the end products of fermentation. For example, when *Streptococcus bovis* grows slowly, it produces acetate and ethanol but, when it grows rapidly, it produces lactate (Ørskov *et al.*, 1990). This species is more acid tolerant than most other rumen micro-organisms.

2.2 Rumen protozoa

The rumen protozoa are more susceptible to acidic conditions than most other rumen micro-organisms (Orpin, 1985). The main purpose of protozoa is to invade newly ingested food, break it down to smaller particles and to store surplus carbohydrates in the form of amylopectin (Ørskov *et al*., 1990). With high-concentrate diets the most significant manner in which the protozoa affect bacterial function is by stabilizing the rumen environment. Certain species, which cannot utilize cellulose, depend largely on starch as their source of energy (Ørskov *et al*., 1990). Their rapid uptake of starch and soluble carbohydrates that enter the rumen restricts the rate of lactic acid formation and this limits the fluctuations of rumen pH.

2.3 Carbohydrate metabolism in the rumen

Carbohydrates contribute 70-80% of the diet dry matter (DM) while protein, fat and minerals make up the remaining part (Hutjens, 2002). Carbohydrates are the primary energy source for the cow and support rumen function and microbial growth. They can be divided into two categories in feed: non-fibre carbohydrates (NFC) where sugar, starch, organic acids, and other reserve carbohydrates such as fructans make up the NFC fraction and are major sources of energy for high producing dairy cattle (NRC, 2001) and structural carbohydrates (SC) (cellulose, hemicelluloses, lignin, and pectin). The rumen microbes digest the sugar, starch and fibre and convert it, amongst others, to VFA. The VFA's produced by the micro-organisms are the main source of energy available for the ruminant. When the VFA ratios and levels shift due to different nutritional factors, they can have an impact on the milk and milk component yields. (Hutjens, 2002). The availability and digestibility of cell wall and cell solubles vary, depending on the growth stage and maturity of the forages, the source of carbohydrates (starch or cellulose) and the processing (grinding of grain or chopping of forages). Dairy farmers and nutritionists have to make decisions on the correct source and rate of starch fermentation in the rumen, based on rumen pH, forage source, level of NSC, dry matter intake (DMI), and cost of starch containing grains.

2.4 Volatile fatty acid

Volatile fatty acids are end products of microbial digestion, and are absorbed from the rumen to serve as a main source of energy for the ruminant. The primary VFA is acetate which represents 55-70% of the total VFA production (Hutjens, 2002). Acetate is produced mainly from the digestion of fibre. Propionate or propionic acid is produced mainly by starch- and sugar-digesting bacteria. Propionate is converted to glucose by the liver and is then, for example, used to synthesize milk lactose. The level of propionate varies from 15-30% of the total VFA production (Hutjens, 2002). The third most prevalent VFA is butyrate and contributes 5-15% of the VFA produced. When evaluating VFA patterns, the ratio of acetate to propionate (2.4:1) reflects the rumen fermentation pattern (Hutjens, 2002). For example, high levels of acetate can indicate a high fibre-low fermentable carbohydrate ration.

2.5 Rumen pH

Rumen pH fluctuates throughout the day and that could have an important effect on the fermentation and digestion in the rumen. Different rumen micro-organisms are active at different pH levels. The growth of fibre digesting bacteria, for example, is favoured when rumen pH is between 6.0 and 6.9 while the growth of starch digesting bacteria is favoured by a pH from 5.5 to 6.0 (Hutjens, 2002). Thus, the high producing cow has to maintain a pH of near 6.0 for the optimal growth of all bacterial populations, which will result in a favourable VFA pattern and yield (Hutjens, 2002).

Several factors affect changes in rumen pH (Mason, 1999):

- The type of diet can cause a shift in the pH, with forage rations usually resulting in a pH of greater than 6. Forage (fibre) stimulates a higher rate of saliva production and secretion. Saliva contains bicarbonate, which helps with buffering the rumen environment.
- The physical form of feed (ground, pelleted or chopped) will affect the size of the feed particles. If the forage particle size is too short, the forage mat necessary in the dorsal rumen cannot be maintained. Fibre digestion will decrease and rumen pH is lowered. Saliva production is also reduced due to less cud chewing time. If concentrates are ground too finely, starch is exposed too rapidly to microbial digestion and increased degradation. The rumen pH drops and propionic

acid and lactic acid production increases. Steam rolling, pelleting or grinding will change starch structure, which makes it more available in the rumen for fermentation.

- The level of feed intake changes the rumen degradation and synthesis. Rumen pH can drop as more substrate, such as starch, becomes available for microbial use, thus increasing acid production. The amount of saliva produced per unit of dry matter can also decline with a drop in DMI.
- Wet rations can reduce rumen pH due to less saliva production and rumination time. If the wet feed is silage, less chewing is needed to reduce particle size, lowering rumination time. If the total ration moisture exceeds 50 % due to ensiled and fermented feeds, DMI can be reduced.
- The method of feeding may change the rumen environment. Total mixed rations (TMR), for example, may stabilize the rumen pH more than feeding concentrate and roughage separately by minimizing the feed particle selection, synchronizing degradable protein and fermentable carbohydrate availability and increasing the DMI.

Although ruminal pH is a critical factor in indicating metabolic acidosis, it should be realised that it is not the only factor (Nocek *et al*., 2002).

Chapter 3

Literature Review

Ruminal acidosis

Ruminal acidosis can be defined as an array of biochemical and physiological stresses caused by the rapid production and absorption of ruminal organic acids and can have a significantly noticeable affect on the production and financial aspect of any intensive high producing dairy or feedlot (Krehbiel *et al*., 1995). There is no local data available on the occurrence of and financial losses due to rumen acidosis. In the USA, the incidence of rumen acidosis is ± 30% and the financial loss has been reported to be $ 1.71 per animal per day (Stock & Britton, 1996).

In this chapter the mode of action, occurrence, symptoms and prevention of sub-acute and acute rumen acidosis will be discussed to provide an insight into the problem of rumen acidosis.

3.1 Mechanism

The events leading to rumen acidosis occur when the animal's diet is suddenly changed from a mainly forage to a high concentrate diet (high in starch or other rapidly fermentable carbohydrates), or when it is fed excessive amounts of such concentrates (McSweeney & Mackie, 1997). The introduction of starch into the rumen, or a sudden increase in the starch supply, leads to rapid fermentation and an increased production of VFA as well as lactic acid, (Plaizier, 2002). Furthermore glucose, which is normally found in very low concentrations in the rumen, is produced from starch or other rapidly fermentable carbohydrates, which results in an increase in ruminal glucose concentrations.

Due to this increase in glucose in the rumen, organisms such as *Streptococcus bovis* and other lactic acid producing organisms, for example *Lactobacillus plantarum,* grow to such an extent and, with an increase in ruminal osmolarity, further increases ruminal acidity by inhibiting VFA absorption from the rumen

(Galyean, 2001). As the rate of VFA and lactic acid production exceeds their rate of removal or conversion, rumen pH may fall below 6.0 (Plaizier, 2002). If the drop in rumen pH is short-lived and the pH rises shortly afterwards, the rumen bacteria can tolerate the change with no significant reduction in cell numbers (Van Kessel & Russell, 1996). *Streptococcus bovis* and other lactic acid producing bacteria are more tolerant to these acidic conditions in the rumen and will proliferate and produce more lactic acid. They start to replace the other normal rumen micro-organisms due to their higher growth rate and become the major component of the micro-organism population in the rumen (Dawson *et al*., 1997). At the same time, organisms that would normally prevent lactate accumulation by metabolising the lactate to VFA's, for example *Megasphaera elsdenii*, are being inhibited.

Lactic acid is a much stronger acid compared to the VFA's and the pH drops further until even the *S bovis* can no longer grow and *Lactobacilli* then start to become the main lactic acid producers. They further ferment the starch to produce more lactic acid until the pH in the rumen declines to below 5.0. This whole process may be very rapid and the overgrowth by lactic acid producing bacteria can occur within 24 h (Dawson *et al*., 1997). Lactic acid, which is normally very low in concentration, can increase to ± 100 mM in severe cases of acidosis. DeFrain *et al*. (2002) found that concentrations of ruminal lactate were undetectable before a SARA challenge and increased linearly ($P < 0.05$) with repeated challenges. A reduction in ruminal pH leads to a rumen microbial shift from lactic acid fermenters to lactic acid producers (Goad *et al*., 1998). Horn *et al*. (1979), however, indicated that increases in ruminal VFA concentrations depress ruminal pH during SARA rather than lactic acid.

During rumen acidosis the ciliate protozoa population, which normally has a stabilizing effect on the ruminal fermentation process, declines because the ruminal pH is below their optimal growth requirements (McSweeney *et al*., 1997). Rumen ciliates disappear if the pH rises above 7.8 or falls below 5.0 (Clarke, 1977).These protozoa have an important role in regulating the production of lactic acid and VFA in the rumen. The protozoa ingest starch, soluble sugars and bacteria, thus reducing the rate of fermentation in the rumen. These organisms are also involved in the metabolism of lactic acid; therefore,

if the micro-organism population is unstable, it will contribute to the severity of lactic acid acidosis (McSweeney *et al*., 1997).

From the previous discussion it can be concluded that rumen acidosis cannot be defined as purely a low ruminal pH, but it can best be described as a syndrome related to a fermentation disorder in the rumen.

3.2. Sub-acute rumen acidosis (SARA)

3.2.1 Causes and Symptoms

Acidosis is categorized as acute or sub-acute, primarily on the basis of the presence or absence of different symptoms. Sub-acute rumen acidosis is also referred to as chronic acidosis. Formulating energy-dense diets to meet the nutritional requirements of high producing dairy cows can promote SARA (DeFrain *et al*., 2002). To detect SARA is not as easy as detecting acute rumen acidosis. The only symptoms that are normally seen are the decrease and / or erratic feed intake. Some additional animal signs of SARA may be reduced milk production, panting, excessive salivation, kicking at the belly, eating dirt, and diarrhoea (Stock & Britton, 1996).

Other symptoms that are not always visible but could be measured include the following (Hall, 1999):

- Reduction in ruminal pH.
- Rumen hypermotility or stasis.
- Reduced rumination (cud chewing).
- Faeces in the same feeding group vary from firm to diarrhoea.
- Faeces foamy, contains gas bubbles.
- Appearance of mucin / fibrin casts in faeces.
- Appearance of undigested fibre in faeces.
- Increase in fibre particle size (>1.25 cm) in faeces.
- Appearance of undigested, ground (< 0.64 cm) grain in faeces.
- Reduced feed efficiency.
- Reduced production compared to that which the ration should support.

- Cows experience laminitis and hoof problems, especially first lactation and fresh cows.
- Hoof surfaces have horizontal ridges or lines.
- Low percentage milk fat.

In group fed animals, the symptoms are very often difficult to observe in individual animals. Whereas acute acidosis is easier to detect and, if diagnosed early, can be treated directly, SARA is probably the most prevalent form, and more difficult to detect and treat (Henning, 2004). There are two types of SARA. Firstly, fresh cow acidosis can occur from seven days pre-partum to 20 days postpartum and is related to a lack of a transition diet and/or rumen problems at calving. Adapted acidosis affects cows 40 to 150 days in milk. Rumen adaptation should have occurred, but these cows receive diets that are short in fibre, high in starch or feeding systems allowing for feed selection. Both types of acidosis can occur and require different strategies to correct (Mason, 1999).

Any interruption of the normal consumption pattern of cattle can cause acidosis. For example, bad weather can disrupt feed intake by causing cattle to consume a greater amount of feed before and after the storm (Stock & Britton, 1996). Other environmental effects include mud and heat. Mud and heat stress reduce feed intake and alter intake patterns. Extreme heat conditions may force cattle to consume a greater proportion of their feed at night, rather than during the day (Stock & Britton, 1996).

3.2.2. Ruminal microbial and fermentative changes associated with sub-acute acidosis

Microbiological changes in the rumen associated with acute acidosis and an adaptation to high-concentrate diets have been well documented (Slyter, 1976; Mackie *et al*., 1978; Mackie & Gilchrist, 1979). However, microbiological changes in animals experiencing SARA were not previously determined prior to 1974. The proportion of lactic acid producing and -fermenting bacteria in the rumens of animals adapted to high-hay or high-concentrate diets is different (Latham *et al*., 1974; Mackie & Gilchrist, 1979). Thus the potential responses and ruminal changes to overfeeding will be different, depending on the diet and production system.

Goad *et al*. (1998) conducted a study to determine the effect of SARA on ruminal microbial and fermentative changes in the rumen. Ruminally canulated steers were fed either a 20% grain (hay adapted) or 80% grain (grain adapted) diet. The ruminal pH in both the hay- and grain adapted steers declined to between 5.0 and 5.5, a pH range that is associated with SARA, as published in a number of studies (Horn *et al*., 1979; Harmon *et al*., 1985; Burrin & Britton, 1986; Stock *et al*., 1990; Krehbiel *et al*., 1995). It is clear that low rumen pH values may even occur on diets not considered as an acidosis risk. Decreased ruminal pH in both hay- and grain-adapted steers was due primarily to an increased VFA concentration. An increased VFA concentration, rather than lactate accumulation, is associated with a decreased ruminal pH during SARA. (Horn *et al*., 1979; Burrin & Britton, 1986). Nagaraja *et al*. (1998) reported that increases in total VFA concentrations are often observed in animals experiencing SARA.

A shift towards increased propionate and butyrate proportions relative to acetate have been reported to occur when feeding grain diets to sheep and cattle and during the onset of sub-acute acidosis (Horn *et al*., 1979, Harmon *et al*., 1985; Burrin & Britton, 1986).

There is also a change in the rumen micro-organism population during SARA. As the ruminal pH dropped below 6.0, rumen *Lactobacillus* numbers increased, because of the more favourable acid environment (Slyter, 1976) with increases in ruminal lactate concentration. Dirksen (1970) suggested that increased total bacterial and Gram-positive bacterial numbers were associated with sub-acute acidosis. Increased numbers of Gram-positive bacteria and ruminal *Lactobacilli* were observed during adaptation to grain feeding (Olumeyan *et al*., 1986).

Protozoa populations decreased extensively with increased ruminal acidity, but complete defaunation did not occur in either dietary groups (Slyter, 1976). Less rapid decreases in total protozoa numbers in hay-adapted steers may have been the result of slightly higher ruminal pH compared with grain-adapted steers or possible other undetermined factors such as ruminal osmotic pressure (Slyter, 1976).

3.2.3. Prevention of SARA

The diagnosis of SARA, before it has an economic impact, is difficult, therefore the prevention of SARA is the best approach. Recommended feeding guidelines and practices for prevention of SARA are as follows (Shaver, 1998):

- Meet or exceed dietary fibre minimums of 18-21% acid detergent fibre (ADF) (DM basis) and 27-30 % neutral detergent fibre (NDF) (DM basis).
- Meet or exceed dietary NDF from forage minimums of 18-21% (DM basis) for hay crop silage based diets and 21-23% (DM basis) for maize silage based diets.
- Do not exceed 35-40% NFC (DM basis).
- Provide TMR with 8-10% of as-fed particles on the top screen of the Penn State/ forage particle size separator.
- Evaluate and regulate the rate of ruminal starch fermentability by manipulating the grain moisture content and fineness of grind.
- Monitor and prevent over-mixing or over-processing of the TMR.
- Monitor and minimize separation during feed mixing and delivery.
- Routinely check the moisture content of wet feeds and adjust rations accordingly to ensure the correct DM ratio of forage to concentrate.
- Feed close-up dry cow 35-40% NFC diets (DM basis) to adapt the rumen micro-organisms population and develop the rumen papillae prior introducing the high-producing group diet.
- Feed a post-fresh transition diet that contains more total NDF and NDF from forage than the high-group diet; this diet may benefit from the addition of baled hay.

3.3 Acute rumen acidosis

The general term “acidosis” is often used to refer to acute rumen acidosis. It is also referred to as lactic acid acidosis. It’s important to know that SARA and acute rumen acidosis are not two different diseases, but that SARA can easily develop into acute rumen acidosis.

3.3.1 Mechanism and symptoms

The symptoms of acute rumen acidosis are anorexia, rumen stasis, rumenitis, diarrhoea, dehydration, laminitis and liver abscesses (McSweeney & Mackie, 1997). The rumen pH will drop to around 5.5 and the lactic acid concentration in the rumen will rise (McSweeney & Mackie, 1997). The ciliated protozoa will reduce or, in severe cases, be eliminated (Henning, 2004). The lining of the ruminal wall can be damaged, and abomasal and intestinal linings can be severely inflamed. Destruction of papillae in the rumen and damage to the linings of the intestines may result in poor absorption of nutrients, resulting in low gains and poor feed efficiencies. Most of the problems associated with acute acidosis can be minimized with proper feed-bunk management. Many cattle diagnosed as “sudden death “may have died from acute acidosis (Stock, 1996). The major differences between acute and sub-acute acidosis are shown in Table 3.1 and the development of acute ruminal lactic acidosis on high starch diets is illustrated in Fig 3.1.

Table 3.1 Comparison of acute and sub-acute acidosis. Nagaraja *et al*., (1998)

Item	Acute acidosis	Sub-acute acidosis
Clinical signs	Present	Absent
Systemic acidosis	Present	Absent
Mortality	Yes	No
Ruminal pH	< 5.0	5.0 - 5.5
Ruminal acids:		
Lactic acid	High (50 - 100 mM)	Normal (0 - 5 mM)
VFA's	Below normal (< 100mM)	High (150 - 200 mM)
Ruminal bacteria:		
Lactic acid producers	Very high	Normal to small increase
Lactic acid utilizers	Significant reduction	Increase
Ruminal ciliated protozoa	Absent or significant reduction	Absent or significant reduction

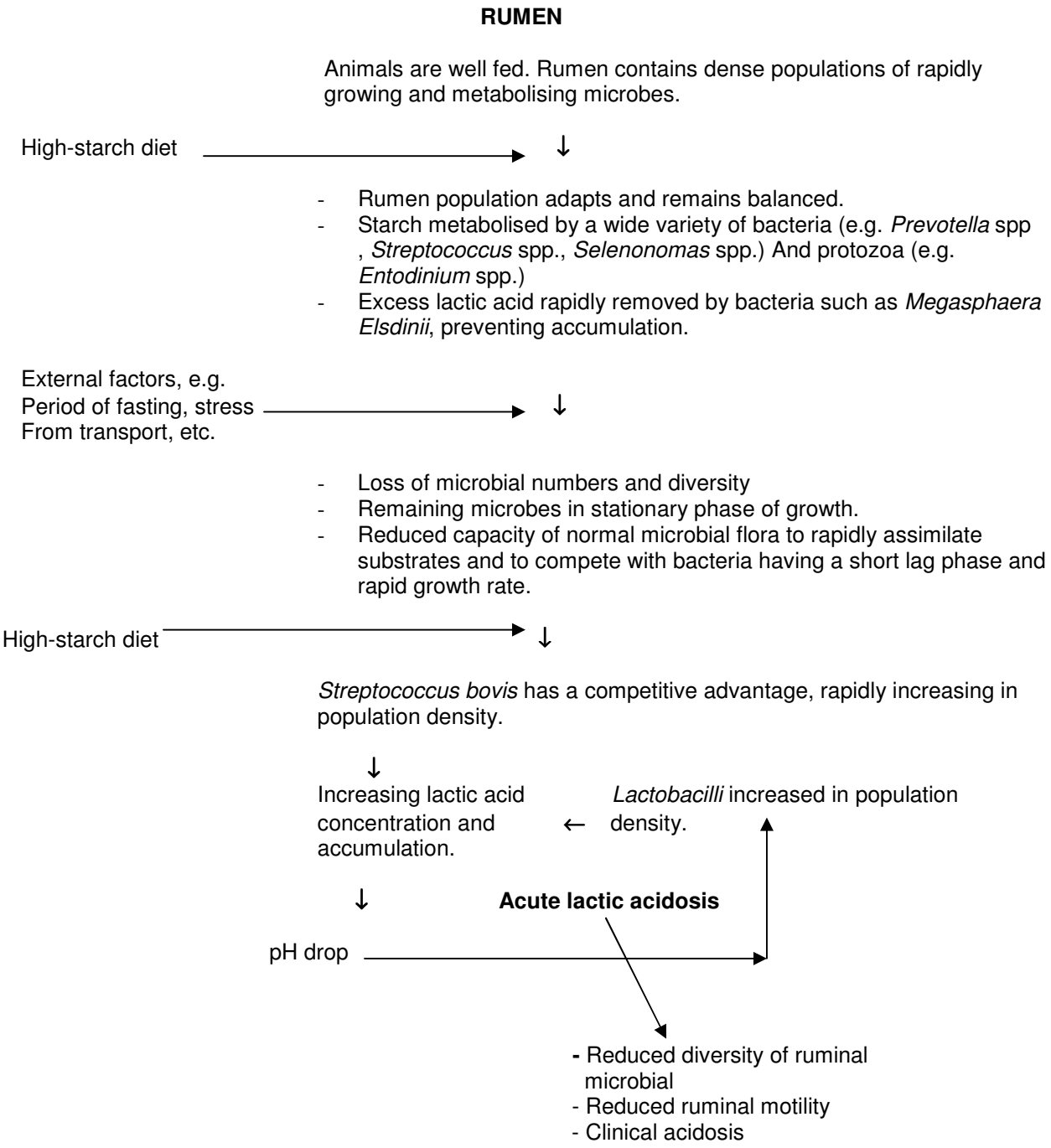


Figure 3.1: Development of acute ruminal lactic acidosis on high-starch diets (Mackie *et al.,* 2002).

3.3.2. The effect of ruminal acidosis on VFA absorption

Ruminal acidosis can have a significant effect on the absorption of VFA's. Several studies have been conducted to determine the effect of acidosis on VFA absorption. Krehbiel *et al*. (1995) examined the effects of increasing severity of rumen acidosis on rates of absorption of ruminal VFA's in sheep. Sheep were dosed with different levels of glucose to induce rumen acidosis. Results have shown acetate, butyrate and isovalerate concentrations to be decreased with an increase in glucose levels. Propionate and valerate concentrations increased as the ruminal glucose dose increased. D(-) lactate and L(+) lactate accumulate with an increase in glucose levels. The increased concentrations of D(-) lactate and L(+) lactate were accompanied by a simultaneous decline in the concentration of total VFA. In support, Harmon *et al.* (1985) observed elevated levels of D- and L-lactate levels during acute acidosis with peak concentrations of 47 and 77 m*M*, respectively, and low levels (< 5m*M*) of D- and L-lactate during sub-acute acidosis.

Acute acidosis impairs ruminal VFA absorption, but the magnitude of the impairment is not clear. Harmon *et al.* (1985) showed that the net portal absorption of VFA in cattle having acute acidosis was 50% less than the VFA absorption in cattle with sub-acute acidosis. However, the lower net absorption of VFA in acute acidosis situations reflected lower ruminal VFA concentrations.

These and other results suggest that the ability of the rumen to absorb VFA may be reduced for an extended period of time in ruminants experiencing rumen acidosis. Abnormal keratinisation of the ruminal epithelium as a result of rumen acidosis may affect the absorption of VFA from the rumen (Fell & Weekes, 1975; Huntington & Britton, 1979). Because the absorption of VFA accounts for 65-75% of the total ME supply in ruminants, (Bergman, 1990) reduction in VFA absorption may reduce the gain and efficiency in production (Krehbiel *et al*., 1995).

3.4 Faeces as a potential indicator of ruminal acidosis

3.4.1. Increase in particle size / undigested material in faeces

When large particles or a large amount of ground grain is found in the faeces, it suggests that the feed is not being retained in the rumen for a sufficient period to be fermented by the micro-organisms. The depression in ruminal digestion may be related to low pH (Strobel & Russell, 1986). An inadequate fibre mat may not retain large particles effectively in the rumen. Both of these situations can be related to inadequate effective fibre (eNDF) intake. The eNDF is the fibre component in the ration that enhances rumination and rumen motility (Hall, 1999).

3.4.2. Mucin / Fibrin casts or gas bubbles in faeces

When feed is fermented in the rumen, the organic acids are absorbed across the rumen wall and the gas (carbon dioxide and methane) is eructed or belched out by the cow. Gas produced from hindgut fermentation can appear as bubbles in the faeces (Hall, 1999). However, a major difference between the hindgut and the rumen is the potential for the fermentation to be buffered against a lower pH. Rumination and mixing with saliva provide buffers to reduce the extent of the pH decline in the rumen (Hall, 1999). However, no system of that magnitude exists for the hindgut. When a large amount of fermentable carbohydrates reaches the hindgut due to a low mean retention time in the rumen, fermentation of carbohydrates to organic acids can result in injury to the gut. The increased acidity and action of the organic acids on the cells of the large intestines can result in a disruption and breakdown of the surface cells or epithelium (Hall, 1999). When the damage is severe, the intestines secrete mucous or fibrin to protect the cells (Argenzio *et al.*, 1988). The gut can repair itself in a few hours to a day depending upon the severity of the damage (Argenzio *et al.*, 1988). The mucin / fibrin casts found in the faeces often have the tubular form of the gut wall cells, suggesting that intestinal damage has occurred. Damage to the large intestines, therefore, may play a role in causing the diarrhoea often seen with rumen acidosis.

3.5. Prevention

3.5.1. Traditional prevention practices

The saying “Prevention is better than cure.” is especially relevant when it comes to acidosis. This is the reason that more emphasis should be put on the prevention of rumen acidosis. The most obvious strategy to prevent acidosis is to ensure that feeding and management are of a high standard. It is important to make sure that the animals are gradually exposed over time from a roughage diet to a concentrate diet (Mackie *et al*., 2002; Shu *et al*., 1999). This is to allow time for the bacteria which are responsible for utilizing lactic acid, and other bacteria that ferment starch, to keep pace with the growth of *S bovis*, thus preventing acidosis from occurring (Mackie *et al*., 2002). If the adaptation period becomes too long, it might impact negatively on milk production. Dairy producers are faced with the challenge to adapt the rumen as soon as possible to handle the high-energy diet and, on the other hand, there is the need to push the energy content of the diet as high as possible in the shortest possible time to obtain optimal milk production (Henning, 2004).

Stimulating the production of saliva is vital in helping the prevention of rumen acidosis since saliva contains buffers such as sodium bicarbonate. The more the cow ruminates the more saliva is produced (Plaizier, 2002). Rumination is stimulated by the fibre content and the particle size (coarseness) of the forage, with coarser diets more effective in stimulating rumen buffering and preventing rumen acidosis (Plaizier, 2002). However, excess coarseness will reduce feed intake. It is, therefore, very important to measure the coarseness of the forages and the diets using the Penn State Forage Particle Size Separator (Plaizier, 2002). Suggested guidelines when using the Penn Sate Particle Size Separator are shown in Table 3.2.

Table 3.2 Recommended particle size distribution when feeding maize silage, haylage or TMR (Townsend, 2000)

	Maize silage	Haylage	TMR
Top pan	5%	>20%	>10%
Middle pan	>50%	20-50%	30-50%
Bottom pan	<50%	<40%	<50%

3.5.2. Buffers

The primary goal when feeding buffers is to stabilise rumen pH. Buffers, such as sodium bicarbonate (SB) and sodium sesquicarbinate are commonly used in dairy diets (Hall, 1999). Buffers do not eliminate the root causes of rumen acidosis, but play a role in buffering the rumen against pH changes (Hall, 1999). Both have been shown to improve milk fat percentage and/or milk yield. In a review of 41 studies where sodium bicarbonate was supplemented in dairy cattle rations (0.4 – 1.7%), containing on average 57% concentrates, varying responses were shown, depending on the base forage fed (Staples & Lough, 1989). When maize silage was the main forage, cows produced an average of 3.6 kg more milk and 0.22% higher milk fat with sodium bicarbonate supplementation. With grass/legume or hay, results were inconsistent. Another theory on the mode of action postulates that when buffers are fed, cattle increase their water intake (Hall, 1999). This increases the ruminal dilution rate which, in turn, increases the flow of liquid and undegraded starch from the rumen. It is recommended that buffers be fed at 0.6 – 0.8% of DMI or 1.2 – 1.6% of a concentrate mixture (NRC, 2001). When evaluating buffers, acid buffering capacity is used to express the amount of acid required to produce a one-unit change in pH (Keunen *et al.*, 2003).

3.5.3. Fibre

It is important to feed the adequate amount and type of fibre. However, “adequacy” can be difficult to define. Adequacy of dietary fibre is determined by the interaction of the cow and the ration. The NRC (1989) general recommendations for lactating cow diets are NDF as 28% of the ration dry matter, and that 75% of the NDF are supplied from forage. These guidelines are subjected to change depending upon fibre form and source. Fibre effectiveness relates to its particle size, digestibility, density and hydration amongst others (Moony & Allan, 1997). The effectiveness of a fibre source can even vary depending upon the characteristics of the other feeds in the ration (Moony & Allan, 1997). If cows can select different parts of the feed that they consume, any estimation of eNDF intake will be poor.

The following guidelines for fibre are recommended by Boman (2001), to minimize and prevent rumen acidosis:

- Feed properly balanced ration that supplies sufficient effective fibre.
- Do not over mix and over process the TMR.
- Provide long stemmed hay or fresh TMR as the cows leave the milking parlour.
- Feed numerous times during the day and increase feed often to encourage more uniform consumption and prevent sorting.
- Be sure when feeding fast fermenting feeds such as wheat, barley and steam flaked grains, that slower fermenting by-product feeds are included to provide balanced rumen fermentation.

High fill diets, which are formulated to provide adequate physically effective fibre to maximize buffering activity by stimulating rumination and salivation, often limit the animal's nutrient intake capabilities and result in decreased milk production (Varga *et al.*, 1984). Diets high in NDF can be formulated using non-forage fibre sources such that the starch content is lower than in diets containing primarily forage NDF. Potentially, this could alleviate the negative effects of starch on fibre digestion (Mertens & Loften, 1980). Most non-forage fibre sources do not stimulate chewing activity (Clark & Armentano, 1997), potentially subjecting the cows to SARA. Soybean hulls are a non-forage fibre source readily available in the USA, although not in SA. Soybean hulls provide a highly digestible source of structural fibre with minimal lignin content (Garleb *et al.*, 1988). However, compared with forages, soybean hulls do not stimulate rumination and can lead to reduced ruminal pH (Weidner & Grant, 1994).

3.5.4. Non-Fibre Carbohydrates

It is important to control the level and type of NFC in the ration to prevent rumen acidosis. The NFC includes organic acids, sugars, starch and soluble fibre such as pectic substances (Hall, 1999). To prevent rumen acidosis, the NFC is restricted to 35% to 40% of ration DM when the NFC is largely sugar

or starch, or 40% to 45% when the other carbohydrates predominate (Hoover & Miller, 1995). Different NFC differs in their effects on ruminal pH. Sugar and starch may ferment to lactic acid, which is a ten-fold stronger acid than acetic, propionic, or butyric acid. Soluble fibres, such as pectic substances, ferment rapidly, but their fermentation is depressed at a lower pH (Strobel & Russell, 1986). The acid contribution of these substances may, therefore, be reduced at a lower ruminal pH. When formulating rations, the different NFC types rather than total NFC should be considered. The effect of the quantities of NFC fed varies with their rate of fermentation. Rapidly fermenting carbohydrates, such as sugar, soluble fibre and some starches, have the potential to decrease ruminal pH rapidly due to the high amount of organic acids present to the rumen in a relatively short period of time (Hall, 1999). It is also important to keep in mind when formulating diets that there is an interaction between the minimum forage NDF and maximum dietary NFC. When the minimum forage NDF % decreases below 19% of DM with one percent, the maximum dietary NFC should decrease with two percent (NRC, 2001). Processing of feed, such a heat and pressure processing, reduction in particle size, or high moisture ensiling, increases gelatinisation, physical availability and the digestibility of grains (Reinhart *et al*., 1997).

3.6. Other means of preventing and controlling rumen acidosis

As stated above, practices to reduce the risk of lactic acidosis in livestock centre around management techniques that are based on introducing grain gradually. This process, however, is time consuming and complicates the feeding management. This also reduces the potential production and efficiency.

3.6.1. Effect of Ionophores on rumen acidosis

Ionophores are lipophilic compounds that are toxic to many bacteria, protozoa, fungi, and higher organisms (Russell & Strobel, 1989). It has been reported that they have a variety of beneficial effects in ruminants. The primary effect of monensin, an ionophore, is through selectively altering the balance of ruminal microbes (Bergen & Bates, 1984). Ionophores inhibit the growth of gram-positive bacteria, for example *S. bovis* and *Lactobacillus,* by penetrating biological membranes and subsequently altering the flux of ions from and into the cell (Bergen & Bates, 1984).

The supplementation of monensin in ruminant diets has been reported to increase ruminal production of propionate (Bergen & Bates, 1984; Weiss & Amiet, 1990) and reduce methanogenesis (Armentano & Young, 1983). Monensin inhibits lactate-producing bacteria and the resulting reduction in ruminal lactate concentration raises the ruminal pH (Russel & Hino, 1985; Callaway & Martin, 1997). Cooper and Klopfenstein (1996) conducted a study in which they monitored ruminal pH and feed intake continuously in beef steers on high grain diets. Monensin was effective in elevating the average ruminal pH and decreasing the area of ruminal pH below 5.6. Ruiz *et al*. (2001) did not detect an increase in ruminal pH in monensin-treated dairy cows fed high concentrate diets.

Mutsvangwa *et al*. (2002) conducted a study where they determined the effects of a monensin controlled-release capsule on diluting sub-acute ruminal acidosis in dairy cows. Monensin supplementation had no effect on ruminal pH under conditions of SARA. Several studies with monensin supplementation have reported a shift in rumen fermentation, as demonstrated by changes in VFA patterns, in dairy cows (Sauer *et al*., 1989). In these studies, animals supplemented with monensin had consistently higher ruminal concentrations of propionate, but changes in ruminal concentrations of acetate and butyrate were not consistent. Green et al. (1999) found the same VFA concentrations pattern with transition dairy cows fed similar diets. Haimoud *et al*. (1995), however, reported that monensin premix did not affect total VFA levels in lactating dairy cows and in beef steers (Muntifering *et al*., 1980; Armentano & Young, 1983).

The effect of ionophores on altering the rumen fermentation results in a possible increase in the supply of nutrients, particularly propionate, to the cow. As a result, the energy balance of lactating cows is improved, thus enhancing, in turn, milk production and the efficiency of milk production (Ipharraguerre & Clark, 2003). The magnitude of these effects, however, appears to be determined by several factors, many of which are poorly understood. Therefore, more research is needed to improve the usefulness of ionophores for lactating dairy cows.

3.6.2. Direct-fed microbials

Direct-fed microbials (DFM), also called probiotics, refer to micro-organisms, generally alive that are usually given to animals in their feed. They can improve animal performance in some way or another (Kung, 2001).

Many commercially available DFM for cattle contain lactate-producing organisms from the *Lactobacillus* genus (Kung, 1999). The original concept of feeding bacterial DFM to livestock was based primarily on potentially beneficial post-ruminal effects, including an improved establishment of beneficial gut microflora (Beauchemin *et al*., 2003; Fuller, 1999). Although the mode of action of DFM in the rumen is not completely understood, it is thought that the presence of lactate-producing bacteria help the ruminal microflora to adapt to the presence of lactic acid (Ghorbani *et al*., 2002), whereas the presence of lactate-utilizing bacteria is thought to prevent the accumulation of lactate (Nisbet & Martin, 1994; Kung & Hession, 1995). Nocek (2002) also stated that DFM may prevent a decline in rumen pH by decreasing lactic acid production and increasing the utilization of lactic acid by some micro-organisms. He reported a reduced risk of acidosis in dairy cows that were fed a combination of lactate-producing bacteria, *Lactobacillus* and *Enterococcus,* due to the fact that the presence of these bacteria caused the rumen micro-flora to adapt to the presence of lactate within the rumen.

There are specific strains of microbials that, when combined, could manipulate and regulate different sections of ruminal metabolism (Nocek *et al*., 2002). It is possible that, when incorporated into the diet, certain DFM combinations that synthesize lactic acid may sustain a level of lactic acid in the rumen that would be higher and less variable. This would stimulate lactic acid utilizing bacteria, which would reduce the total lactic acid available in the rumen as well as total ruminal acidity (Nocek *et al*., 2002). Cattle diets, supplemented on a daily basis with lactate-utilizing bacteria and / or lactate-producing bacteria, have been shown to improve the feed efficiency and ADG of feedlot cattle (Swinney-Floyd *et al*., 1999; Galyean *et al*., 2000).

There is evidence that supplementing diets with yeast, for example *Saccharomyces cerevisiae,* increases the milk production of dairy cows (Yoon & Stern, 1995). Production responses due to yeast added to the diet are usually related to the stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fibre digestion, and increased flow of microbial protein from the rumen (Martin & Nisbet, 1992; Newbold *et al*., 1996), which may be beneficial for feedlot cattle as well as high-producing dairy cattle fed high-grain diets.

A trial was conducted by Kung *et al*. (1995) to establish whether *Megasphaera elsdenii* (*Me*) (the major lactate-utilizing organism in the rumen of cattle adapted to a high grain diet), would prevent the accumulation of lactic acid in the rumen during the transition from a low to a high concentrate diet. *Megasphaera elsdenii* B159 was grown anaerobically in an overnight culture. A simulation of ruminal lactic acidosis was used. Ruminal fluid was obtained from a fistulated steer fed a lucern-grass hay diet. Ruminal fluid and digesta were collected and strained through four layers of cheesecloth. The particulate-bound micro-organisms were recovered under anaerobic conditions. A buffer containing ruminal micro-organisms, ruminal fluid and a reducing fluid were mixed with a small amount of the diet in flasks. *Me* slurry was added to the buffer mixture. The flasks were incubated anaerobically at 39°C and 2ml of culture media were removed at 0, 2, 3, 4, 5, 6, 7, 8, 10, and 24h of incubation. The samples were analysed for pH (Kung *et al*., 1995).

Above a pH of 5.4, *Me* remains fairly competitive as it was only when the pH fell below 5.4 that the ratio of *S. bovis : Me* increased until *Me* disappeared (Kung *et al*., 1995).

The concentration of acetate and propionate initially increased in cultures inoculated with *Me* but these concentrations decreased after four hours of fermentation. The concentrations of acetate and propionate were lower and the concentration of isobutyrate, butyrate, isovalerate, and valerate were higher when *Me* was dosed compared to the control animals (Kung *et al*., 1995). *Me* prevents high levels of lactate accumulation in the rumen, but the magnitude of the effect depends on the initial dosing rate (Kung *et al*., 1995).

Based on the suppression of lactate alone, lactate accumulation could be prevented in a second dietary challenge without further inoculation with *Me.* This observation requires verification through *in vivo* studies and may be an extremely important finding because multiple dosings, in practice, would be undesirable. The formate and caproic acids concentrations in the rumen fluid can also be measured to verify whether *Me* had an effect because they are end products of *Me.* (Kung *et al*., 1995). *Me* appears to be the major ruminal lactate-utilizer (Kung *et al*., 1995) because *Selenomonas ruminantium* undergoes catabolite repression and is relatively acid-intolerant. *Me* simultaneously use lactate, glucose, and maltose (Russell & Baldwin, 1978) and will compete with lactate producing organisms such as *S. bovis* for substrate. In theory *Me* would be the ideal organism to inoculate into the rumen of animals not adapted to a high concentrate diet in order to prevent the accumulation of lactic acid in the rumen.

Robinson *et al*. (1992) dosed steers intraruminally with *Me* 407A organisms. He reported reductions in lactate accumulation, higher ruminal pH, and a 24% increase in DMI above that of the control animals. Cook *et al*. (1977) dosed crossbred heifers that were unadapted to a high-concentrate diet with cultures of *Me, Peptococcus asacchrolyticus,* and *S. ruminantium.* Cattle were switched from an all-hay to an 85% concentrate diet and dosed with the respective organisms by stomach tube. The animals inoculated with *P. asacchrolyticus* gained more weight than control animals, however, the heifers inoculated with *S. ruminantium* or *Me* gained less body weight than control animals over a 21-day period. Hibbert *et al*. (1993) reported that oral drenching with *Me* improved intake and prevented lactic acid acidosis in cattle when switched from a 50 to 90% concentrate diet.

Propionobacterium also have the ability to utilize lactic acid and may be considered as DFM against acidosis. However, these micro-organisms are probably too slow growing and acid-tolerant to prevent an acute lactic acidosis challenge (Kung, 2001). When a combination of lactate-producing bacteria, *Lactobacillus* and *Enterococcus,* were fed to dairy cattle, a reduced risk of acidosis was reported. It was presumed that they caused the rumen microflora to adapt to the presence of lactate within the rumen (Kung, 2001).

Nocek *et al.* (2002) conducted a trial where a combination of DFM (*Enterococcus faecium* (**EF**), *Lactobacillus plantarum* (**LP**), and *Saccharomyces cereviciae*, a yeast culture), was inoculated into the rumen of fistulated cows. Ruminal pH was measured continuously. The hypothesis was that producers of lactic acid (DFM) would provide a tonic level of lactic acid to stimulate the lactic acid utilizers which, in turn, will increase ruminal pH. This data suggested that there is a level of DFM (10^5 colony forming units/ml (cfu/ml)) that increases ruminal pH. Higher levels of DFM (10^6 to 10^7 cfu/ml) decrease ruminal pH. Apparently acid production overwhelmed utilization at these higher levels. Cows fed 10^5 cfu/ml DFM tended to have a higher digestion rate than those fed the higher DFM concentration levels. Nocek *et al*. (2002) concluded that feeding lactating dairy cattle a specific combination of EF and LP in combination with yeast leads to a significant alteration in ruminal pH for cows fed a high grain diet. Cows fed a concentration of DFM (10^5 cfu/ml) were able to sustain a higher rumen pH than cows fed DFM (10^6 cfu/ml, or 10^7 cfu/ml). Nocek suggested that DFM are producing acid in sufficiently large quantities to stimulate acid utilizers. However, there appeared to be a threshold of acid production that would not support further growth of utilizers.

Ghorbani *et al*., (2002), conducted a study to determine whether DFM could be used to minimize the risk of acidosis in feedlot cattle receiving high concentrate diets. *Propionibacterium* P15 (P15) and *Enterococcus faecium* EF212 (PE) were added to diets on a daily basis with a control diet to compare the results. The mean ruminal pH averaged 5.71 and was not affected by adding DFM to the diet. Ghorbani *et al*. (2002) reported that neither DFM influenced the total VFA, concentrations of propionate, isobutyrate, and isovalerate, or the acetate-to-propionate ratio. Results from this experiment furthermore demonstrated that the effects of DFM, particularly P15, was on the protozoa population. The total protozoa count for steers fed P15 was significant higher than those fed PE ($P < 0.05$) or control ($P < 0.003$). Most of the protozoa populations were identified as *Entodinium.*

Although they hold much promise, responses with DFM in ruminants are often small and highly variable and much research is still needed on different aspects such as dosage size, form of application, for example a single massive dose versus continuous daily dosing and addressing the viability of oxygen

sensitive micro-organisms (Kung, 2001). There is also very limited published information on the mechanisms by which bacterial DFM improved animal performance, particularly for cattle adapted to high-grain diets (Ghorbani *et al*., 2002)

3.6.3. Manipulation of Ruminal fermentation with Organic Acids

Organic acids can be used to manipulate ruminal fermentation by stimulating the growth of prominent ruminal bacteria, for example, *Selenomonas ruminantium.* By stimulating the growth, the mixed ruminal micro-organism fermentation pattern can be altered and the performance of feedlot steers and high producing dairy cattle can be improved (Nisbet & Martin, 1990, 1991, 1993, 1994; Martin & Streeter, 1995; Callaway & Martin, 1996; Martin *et al*., 1999). *Selenomonas ruminantium* is a common Gram negative ruminal bacterium that can account for up to 51% of the total viable bacterial counts in the rumen (Caldwell & Bryant, 1966). When *S. ruminantium* is grown in batch cultures with glucose, homolactic fermentation occurs (Hobson, 1965). When the glucose is depleted from the medium, *S. ruminantium* then utilizes lactate as an energy source (Scheifinger *et al*., 1975) but only some strains of *S. ruminantium* are able to ferment lactate (Stewart & Bryant, 1988). Research showed that *S. ruminantium* strain HD4 requires L-malate, CO_2 *p*-aminobenzoic acid, and biotin for growth in a lactate-salts medium (Linehan et al., 1978). Martin (1998) conducted a study to evaluate the effects of aspartate, fumarate and malate on growth and lactate uptake by *S. ruminantium* HD4. Uptake of D + L lactate was stimulated over ten-fold by L-malate (Nisbet & Martin, 1990).

Limited *in vivo* research studies have been conducted to evaluate the effects of organic acids on ruminant performance. Kung *et al*. (1982) reported that feeding 140 g of malate per day increased milk persistency in lactating cows and increased total VFA during early lactation. Feeding malate to Holstein bull calves improved ADG and feed efficiency but had little effect on blood serum constituents (Sanson & Stallcup, 1984). Therefore, the addition of malate to the diets of ruminants fed high levels of rapidly fermentable carbohydrates may improve the ability of *S. ruminantium* HD4 to utilize lactate at a pH under 6 in the rumen.

3.7. Variable incidences/responses observed in practice and research

There appears to be variation in the incidence of acidosis and in the response observed with different remedies (Henning, 2004). The reason may be the fact that a variety of nutritional, management, genetics, behavioural and environmental factors appears to be involved in the development of acidosis (Galyean, 2001). There is, for example, considerable variation in rumen pH among animals fed the same diet. There is also a large variation in the ability of animals to cope with a given carbohydrate challenge (Galyean, 2001). Variation in feed intake and erratic feed intake has been suggested to increase the incidence of acidosis; however, the exact nature of the relationship involved appears to be unclear due to its complexity (Galyean, 2001). Other factors such as acids in the diet (e.g. when feeding high levels of silage) and a failure to produce sufficient endogenous buffers (e.g. with diets resulting in reduced saliva flow) may also explain the variation in the incidence of acidosis.

Mackie *et al*. (2002) reported that the rapid introduction to a starch-based diet alone is insufficient to trigger an episode of lactic acidosis. They propose that other factors such as the period of feed shortage are required to predispose the rumen and allow *S. bovis* to outnumber other normally dominant bacteria. This may explain those cases where the experimental diets designed to induce acidosis appeared unable to give the desired effect.

3.8. Heat stress

Changes in a cow's behaviour and acid-base balance during heat stress predispose her to rumen acidosis. Heat stress alters a cow's acid-base balance. As a cow pants and exhales carbon dioxide, it often appears that the total amount of buffering capacity within her system is decreased (Hall, 1999). In a study conducted at the University of Missouri, the effect of ambient temperature on rumen environment was investigated (Mishra *et al*., 1970). Lactating Holstein cows were fed high roughage or high concentrate diets at environmental temperatures of 18 °C or 30°C with relative humidity of 50% and 85%, respectively. Ruminal pH was lower at the higher temperature and in cattle fed the higher concentrate ration. There was also an interaction of diet and temperature. Ruminal ammonia and lactic acid concentrations were significantly higher for the high temperature treatment. Others have also reported

decreased ruminal ph in hotter versus cooler environmental temperatures (Niles *et al*., 1998). The changes in the rumen appear to be in response to the environmental temperature and not the ruminal temperature.

3.9. Laminitis

Lameness in dairy cattle is associated with serious direct and indirect economic losses, for example, decreased milk production, increased veterinary costs, loss of body weight, delayed oestrus, silent oestrus, increased open days, and early culling associated with loss of genetic potential (Rathwell, 2000). Laminitis can be expressed as different syndromes such as sole abscesses, white line disease, sole haemorrhage, or sole ulcers. There are numerous factors associated with this disorder, but nutrition is one of the major contributors (Rathwell, 2000). Laminitis is a painful inflammation of the tissue in the inside of the hoof. The tissue is called laminae. Laminitis hinders the cow from standing and walking. Cows eat less and produce less milk. Recent studies have shown that an affected animal's production can drop by as much as 2.8 kilograms per day (Mongeon, 2003).

An episode of rumen acidosis can occur long before signs of laminitis become visible. The rumen bacterial population changes and the fibre digesting organisms tend to die due to rumen acidosis. This whole process leads to the absorption of compounds known as metabolites, endotoxins and histamines into the animal's bloodstream. These compounds can affect the blood supply of the growing hoof wall and often lead to clinical or sub-clinical laminitis (Mongeon, 2003). High levels of blood histamine kill gram-negative bacteria in the rumen and endotoxins are released into the blood stream resulting in a blood pool in the claw of a cow's hoof. Rumen acidosis also produces a toxin that activates a metalloproteinase (MMP). This MMP breaks down the bonds between the epidermis of the hoof wall and the soft tissue in the hoof's corium. This leads to sole ulcers and white line abscesses (Mongeon, 2003).

3.10. Bottom line

It is generally agreed by experts that rumen acidosis represents a real threat when ruminants change from a roughage diet to a high concentrate diet, or when the amount of concentrates being fed is

suddenly increased. There are however, reasons why it may sometimes not be observed (Henning, 2004).

These include:

- SARA occurs more often in ruminating animals than acute acidosis and is often not identified.
- The complexity of the “syndrome” known as acidosis is really a multi-component disorder, with many interactions determining the final observed outcome.
- In acidosis research we often “control too well” the other predisposing factors needed, in addition to the increase in ruminal starch availability, to stimulate acidosis and lactic acidosis.

It is clear that the need remains to take the necessary precautions against acidosis in feedlot and dairy cattle in order to prevent mortalities, to keep treatment costs down and to prevent a loss of production. It is also clear that further research is required before the complexity and mechanism of rumen acidosis will be fully understood specifically. More research is especially needed in the field of development and application of DFM’s (Henning, 2004). Nocek (2002) commented as follows “SARA is a way of life, you can’t cure it, you manage it”. The efficient use of DFM’s would, therefore, greatly contribute to managing SARA.

Chapter 4

Material and Methods

4.1 Introduction

The objective of this trial was to investigate the effects of *Megasphaera elsdenii* NCIMB 41125 (*Me*) on the productivity, health and some aspects of rumen fermentation in lactating Holstein cows. The hypothesis is that the effect of *Me* would be more pronounced on high starch diets and, therefore, two different types of diets were used namely, a 60% concentrate diet, which is the industry norm, and a 70% concentrate diet. The *Me* was produced at the Gastro Intestinal Microbiology and Biotechnology Laboratory of the Livestock Business Division, ARC, Irene. Animals were cared for according to the guidelines for the Care and Use of Animals in Agriculture, Research and Teaching (1999) and animal use was approved by the Animal Use and Care Committee of the University of Pretoria. In this chapter the materials used and methods followed are discussed.

4.2. Animals, diets and experimental design

Sixty multiparous Holstein-Friesian dairy cows were used in a randomized complete block design experiment. Three weeks pre-partum cows were assigned to one of 15 blocks of four cows, based upon parity and previous lactation milk production. The dairy cows were divided randomly into four groups, namely:

Group 1: Low concentrate diet Control group (LCC).

Group 2: Low concentrate diet Dosed group (LCD).

Group 3: High concentrate diet Control group (HCC).

Group 4: High concentrate diet Dosed group (HCD).

The period from 21 days pre-partum until calving was designed as the “steam-up” period and during this period the cows received 7 kg total mixed ration (LCC TMR) without dosing *Me* and with 400 g anionic

salts per day. The anionic salts consisted of a mixture of 200g NH_4Cl_2 and 200g $MgSO_4$. Medium quality *Eragrostis curvula* hay (14% CP; 63% NDF on a DM basis) was available *ad lib*. Once a cow calved, she was moved to a semi-intensive housing unit equipped with Calan head-gates (American Calan Inc., Northwood, NH, USA) for individual feeding. The cow was then allocated to a specific pen where she received her allocated diet and had access to a dirt exercise area of 200 m^2. During the 60 days post-partum experimental period the cows only received their allocated TMR's, without any additional hay. For most of the trial period, there were cows in all 4 the treatments. Both the control and treated groups start and end at more or less the same time with the trial. The TMR was fed *ad lib* once a day after the morning milking (Table 4.1). Feed allocations were monitored daily to ensure 5 – 10% refusals. Thirty percentage water was mixed into the TMR to decrease dustiness and enhance feed intake. The experimental diets were formulated using the CPM-Dairy programme (version 3.0.5, 2006). Fresh water was available *ad lib*. The cows received the *Me* orally on day 2, 10 and 20 post-partum. Preparation of the *Me* dosing is discussed later in this chapter.

The cows were milked three times per day: at 05h00, 12h00 and 19h00 respectively, in a ten-point herringbone DeLaval milking parlour with electronic ID and automatic cluster removal (De Laval (Pty) Ltd, Pinetown, 3600, South Africa). During the first ten days post-partum, rectal temperature was measured at 13:00 daily and rectal examinations were performed from day six post-partum until the cows recovered to confirm that they recovered from calving as expected.

Throughout the trial period cows were monitored for any health problems which were treated accordingly. When any type of health problem affected a reduction in DMI and/or milk production of 30 – 50% of the previous week's production or DMI for more than five days, the cow was replaced with a healthy, fresh in milk cow with the same milk production potential. The reason for the replacement was that it was necessary to determine the effect of the *Me* dosing on the production of healthy cows. Records were kept of all the health problems as well as the numbers of all the cows that were culled from the trial.

Table 4.1. Ingredient and nutrient composition of the experimental diets fed both pre-partum and post-partum (DM basis)

ITEM	Low concentrate	High Concentrate
Lucerne hay	31.9	19.6
Eragrostis curvula hay	7.9	7.9
Maize meal (ground)	35.4	47.9
Molasses	3.9	3.9
Whole cottonseed	7.9	7.5
Soyabean meal	10.2	9.4
Maize gluten 60	1.6	2
Urea	0	0.27
DiCaP	0.79	0.79
Min/Vit Premix [1]	0.39	0.39
Nutrient composition (%DM)		
Roughage	39.8	27.5
CP	18.1	17.8
UDP (%CP)	41.7	41.1
Sol CP (%CP)	25.1	25.9
ME (MJ/kg DM) [3]	11.2	11.9
NDF	28.2	23.8
pe NDF	23.5	18.7
NFC [2]	44.8	50.4
Fat	4.2	4.3
Ca	0.84	0.76
P	0.49	0.49

[1] Contains per kg of premix: 7000 KIU of Vitamin; 1500 KIU of Vitamin D_3; 1300 mg of Vitamin B_1; 4000mg of Vitamin B_{12}; 15,000 mg of Vitamin E; 130,000 mg of niacin; 1000 mg of Co, 3000 mg of I; 375 mg of Se; 100,000 mg of Mn; 20,000 mg of Cu; 100,000 mg of Zn; 350,000 mg of S; 60,000 mg of Fe.

[2] Non-Fibre Carbohydrates (NFC) = 100 – (CP + Fat + Ash + NDF).

[3] Calculated using the database of Van der Merwe and Smith (1991).

4.3. Sample collection, data recording and analyses

4.3.1. Feed samples

The amount of TMR offered and refused was recorded daily. Samples of TMRs were collected every 14 days, frozen at -20℃ and composite by treatment on a monthly basis. Samples of refusals were taken every 14 days, frozen at -20℃ and composite within cow. To determine whether feed selection had occurred, a representative refusal sample was analyzed for NDF and NFC and the ratio of NDF: NFC was compared to that ratio of the respective TMR. Representative samples of TMR were dried at 60℃ for 24

hours before analysis. The following analyses were performed on feed samples: DM according to AOAC (2000) procedure 934.01, CP according to AOAC (2000) procedure 988.05, GE (MC-1000 Modular Calorimeter. Operators manual), EE according to AOAC (2000) procedure 920.39, NDF and ADF according to Van Soest *et al*. (1981), NFC according to Tilley et al. (1963), Ca according to Giron (1973), P according to AOAC (2000) procedure 965.17 and soluble CP according to AOAC (2000) procedure 968.06.

4.3.2. Milk recording and sampling

Milk production was measured on a daily basis for 60 days after calving. Milk samples were taken once a week during the 12h00 milking session. The milk samples were analyzed for milk fat, milk protein, milk urea nitrogen (MUN), and milk lactose using the System 4000 Infrared Analyzes (Foss Electric, Hillard, Denmark).

4.3.3. Rumen fluid sampling and analysis

Rumen fluid samples were taken on day 15 and 30 post-partum between 4 and 5 hours after the morning feeding, using the Rumenocentesis procedure described by Nordlund and Garrett (1994). This involved pushing a needle through the body wall into the rumen and withdrawing ca 10 to15 ml rumen fluid before pulling the needle out again. The pH of the rumen fluid sample was measured within 30 seconds after extraction of sample out of the rumen using a pH meter (IQ Scientific palm pH/mV/Thermometer). The rumen fluid samples were filtered through two layers of cheesecloth to separate larger feed particles. One millimetre of the filtered rumen fluid was stored at -10℃ for lactic acid analysis using a modification of the Barker-Summerson method (Pryce, 1969). Another 4 ml of the filtered rumen fluid was mixed with 1ml of 25% H_3PO_4 (Orthophosphoric acid) to preserve the sample, and then stored at -10° for VFA analysis using the Gas Chromatographic method (Gibbs *et al*., 1973)

4.3.4. Faecal sampling

Faecal samples were taken rectally from all cows on day 15 and 30 post partum, at the same time as the rumen samples. Faecal pH was measured with a pH-meter (IQ Scientific palm pH/mV/Thermometer). After the faecal sample was taken the cow was stimulated by hand internally in the rectum to encourage the excretion of more faeces that dropped to the ground. From the faecal pile, the following could be observed:

- Faecal score (see faecal scoring system),
- Amount of gas bubbles in the faeces,

The faecal samples were stored at -10℃ immediately after collection for later starch analysis. (MacRae *et al.*, 1968)

Faecal Scoring System (Hall, 1999) :

Score	Appearance
1	Very liquid in nature No rings or dimpling Faeces puddles / runs
2	Does not pile Less than 2.5cm deep Appearance of rings
3	Porridge consistency Stand 3.5 cm high 4 - 6 concentric rings / dimples
4.	Faeces are thick Does not stick to shoes No dimpling or rings
5.	Firm faeces balls 5- 10 cm high

4.3.5. Live weight and BCS

Cows were weighed and body condition scored at assignment and thereafter every second week. The five point BCS system (1= very thin, 5= very fat) were used (Wildman *et al.*, 1982)

4.3.7. Incubation and application of *Megasphaera elsdenii*

Me was dosed to the allocated group of cows at 13h00 on day 2, 10 and 20 post-partum. The dosing dates were chosen for the following reasons: On day two post-partum the feed intake of the cows starts to increase significant. On day ten post-partum the second increase in feed intake occurs. The 20 day post-partum dosing was intended to be a back-up dosing. Each dosing contained 250 ml of live 10^9 (cfu/ml) *Me.* The *Me* was prepared 24 hours prior to dosing by inoculating fresh growth medium (200ml) with *Me* culture (50ml) and then incubated for 24 hours at 39°C. The resulting culture was then drawn up into a 300ml dosing syringe and dosed orally to the cow.

4.4. Statistical Analysis

Data were analyzed statistically as a randomized complete block design with the GLM model (SAS, 2001) for the average effects over time. Repeated measures analysis of variance with the GLM model was used for repeated week or perennial measures. Means and Standard Error Means (SEM) were calculated and the significance of difference between means was determined by the Fishers test (Samuels, 1989). Significance was declared at $P > 0.05$ unless otherwise noted.

Chapter 5

Results and discussion

5.1. Introduction

During this trial, a number of parameters were measured to determine whether *Me* had any effect on the productivity, general health and metabolism of the Holstein dairy cow. In this chapter the results of DMI, milk production and composition, BW and BCS, rumen fermentation and faecal parameters as well as health aspects will be discussed. Very little research has been done with *Me* in animal trials especially on dairy cows. The only other production dairy trial of which I am aware was done by L. J. Erasmus (1997). In the discussion the results from this trial will be compared to that of Erasmus as well as other studies where feed additives such as ionophores were used as a preventative measure against acidosis.

5.2. Experimental diets

As expected there were differences in the calculated and actual nutrient content of the experimental diets. The actual and calculated values for CP, NDF, Fat and Ca are shown in Table 5.1. The biggest difference was in NDF with the actual values up to 27% higher. Two factors contributed to this, namely the fact that it is virtually impossible to take a TMR sample that is 100% representative and, secondly, an initial underestimation of the roughage NDF values. This could have an effect on several factors, for example the rumen pH, as more fibre in the diet could increase the rumen pH, but it is important to note that the difference of %NDF between the two diets is similar in the calculated and actual diet.

Table 5.1. Nutrient comparisons of 6 feed samples between calculated and analyzed nutrient composition of the experimental diets, namely low concentrate (3 samples) and high concentrate (3 samples) diets fed to early lactation Holstein cows

ITEM	**Calculated**		**Analysed**	
Nutrient composition (%DM)	**Low Conc**	**High Conc**	**Low Conc**	**High Conc**
CP	18.1	17.8	16.16	16.42
NDF	28.2	23.8	37.65	32.81
EE	4.2	4.3	3.59	3.56
Ca	0.84	0.76	1.08	0.56

5.3. DMI, milk production and feed efficiency

Results in DMI, milk production and feed efficiency are presented in Table 5.2. The average DMI was 23.3 kg/d which is 3.73% of average BW and is considered normal for this stage of lactation (NRC, 2001). A fluctuation of DMI over time is shown in Fig 5.1. After 60 days the general trend was for DMI to increase since DMI only peaks around 10 to 15 weeks post-partum (Erasmus *et al.*,2000).

5.3.1. Dry matter intake

The highest DMI was observed in the HCC group at 24.3 kg DM per day and the lowest at 22.4 in the LCD group. There was no significant difference ($P > 0.05$) in the average DMI between the different treatments (Table 5.2).

Erasmus (1997) observed an average DMI of 22.2 to 24.6 kg/day and Mutsvangwa *et al*. (2002) reported a DMI of 22 to 23 kg per day in early lactating Holstein cows on lucerne based TMR. This is in agreement with results from this study. In theory an increase in DMI for the cows dosed with *Me* would have been expected because a potentially lesser acidic rumen environment was expected, especially in the case of the high concentrate diet groups. This hypothesis, however, did not materialize. There was furthermore no significant difference ($P > 0.05$) in DMI variation between the different treatment groups (Table 5.2). The average DMI variation was 2.9.kg DM/day. DMI variation was calculated by subtracting the previous day's DMI from the present day's DMI.

If the % NDF in the feed orts of the different treatments is compared it is clear that there was no difference in the selection of specific feed particles between treatments, between dosed and control cows or between high and low concentrate diets (Table 5.2). When the average % NDF of the feed orts which is ± 41% is compared to the actual NDF percentage (32 – 38% NDF) of the TMR (Table 5.1), it is clear that a degree of selective eating did take place, the extent of selective eating however was the same for all treatments based on NDF analysis.

There was a significant difference (P = 0.05) in DMI between the high and low concentrate diets (24.0 kg/day and 22.7 kg/day) as shown in Table 5.2. This was expected due to the difference in the NDF levels in the two diets. There was also a tendency towards a higher variation in DMI (P = 0.08) in the high concentrate diet group (Table 5.2).

Table 5.2 The effect of dosing live *Megasphaera elsdenii*, a rumen lactic acid utilising micro-organism, on dry matter intake (DMI) , milk production and feed efficiency of high producing, early lactation, Holstein cows

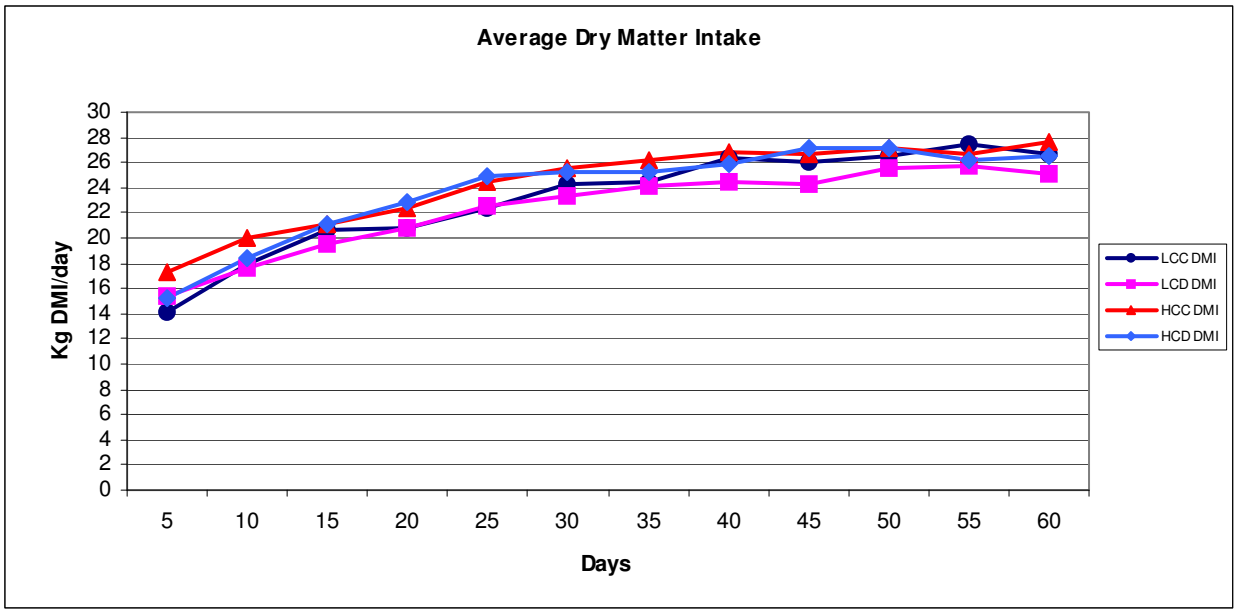
	Treatments [1]						Main Effects					
	LCC	LCD	HCC	HCD	S.E.M. [2]	P =	Control	Dosed	P =	Low Conc	High Conc	P =
Average DMI (kg/day)	23.11[ab]	22.37[a]	24.27[b]	23.81[ab]	0.64	0.83	23.69	23.09	0.36	22.74	24.04	0.05
DMI Variation (kg/day)	2.88	2.69	3.06	3.12	0.17	0.47	2.97	2.91	0.71	2.79	3.09	0.08
DMI:Mass (g DMI/1kg body mass/day)	36.5	36.3	38.5	38.7	1.11	0.88	37.0	38.0	0.98	36.4	38.6	0.06
Feed Efficiency (kg milk/1kg DMI)	1.93	1.94	1.88	1.91	0.06	0.83	1.91	1.92	0.79	1.93	1.89	0.54
Average daily milk production (kg milk/day)	43.9	42.7	45.1	44.2	1.53	0.91	44.5	43.5	0.51	43.3	44.6	0.39
305 days milk production predictions (litre) [3]	12052	11697	11929	11438	329.03	0.84	11991	11568	0.21	11875	11684	0.57
% NDF of Feed orts	42.6	43.1	38.6	39.5	1.34	0.73	40.6	41.3	0.73	42.85	39.05	0.68

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

[1] LCC : Low Concentrate Control; LCD : Low Concentrate Dosed; HCC : High Concentrate Control; HCD : High Concentrate Dosed

[2] S.E.M. : Standard Error Mean

[3] 305 day milk production prediction determined from the standard lactation curves, based on milk production levels, breed, age and calving season (Iris version 33 Intergis 2000 program)



LCC : Low Concentrate Control
LCD : Low Concentrate Dosed
HCC : High Concentrate Control
HCD : High Concentrate Dosed
DMI : Dry Matter Intake

Fig 5.1 The effect of *Megasphaera elsdenii,* a rumen lactic acid utilising micro-organism, on the average dry matter intake pattern of high producing Holstein cows during the first 60 days of lactation.

5.3.2. Milk production and feed efficiency

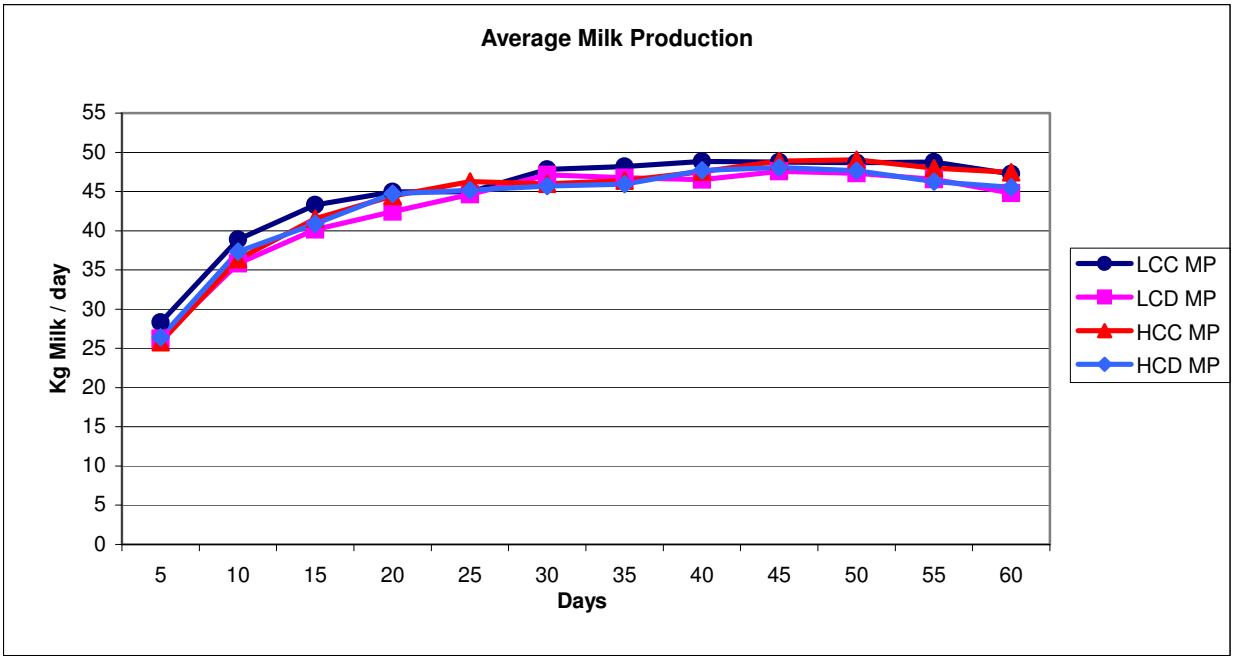
If the treated animals were compared to the control animals there was no significant difference ($P > 0.05$) in average milk production (Table 5.2). The overall average milk production was ± 44 litres milk / day. There was furthermore no significant difference ($P > 0.05$) in total 305 day predicted milk production as would have been expected. The 305 day milk production predictions were calculated using the Milk recording scheme (Iris program version 33, 2006). The level of milk production correlates with other high producing dairy trials (DeFrain *et al*., 2006). Erasmus (1997) observed less milk production at an average of 32 – 36 kg milk/day with two milkings per day but, when only the milk production of the high producers in the trial was used, the average production rose to 39 kg/day.

It is difficult to speculate as to why milk production was not affected by *Me* dosing in this trial. Two factors might have contributed to the lack of response, namely the diet itself and secondly the overall management. Although in the high concentrate diet the maize content was 48% and the NDF level 32.8%, the maize could have been ground too coarsely and the diet did not lack effective fibre, therefore, the pH did not drop sufficiently to induce SARA. The general management in terms of mixing feed uniformly, fibre length, no empty bunks etc. was good and it is possible that differences might have been more pronounced under less than optimal management situations.

Feed efficiency is generally expressed as kg milk/kg DMI and is increasingly being used as a troubleshooting tool and one of the factors that impact on the profitability of the enterprise. The feed efficiency varied from 1.88 – 1.94 and did not differ between treatments ($P > 0.05$) or between high and low concentrate diets ($P = 0.54$). According to Hutjens (2002), cows during early lactation should have a feed efficiency of > 1.8, which is in agreement with this study. Such high feed efficiencies support the suggestion that SARA was probably not present at high sufficiently levels that *Me* could have elicited a response.

The average lactation curves for the four treatments are shown in Fig 5.2. The curves are typical for high producing cows on a TMR i.e. a flatter curve compared to a system where concentrate and roughage are fed separately. In most situations cows would consume more TMR than pastures plus concentrate fed separately (Bargo *et al*., 2002)

Milk production peaked between days 37 and 52 which is typical for high producing cows (Erasmus *et al*., 2000)



LCC : Low Concentrate Control
LCD : Low Concentrate Dosed
HCC : High Concentrate Control
HCD : High Concentrate Dosed
MP : Milk Production

Fig 5.2 The effect of *Megasphaera elsdenii,* a rumen lactic acid utilising micro-organism, on the average milk production pattern of high producing Holstein cows during the first 60 days of lactation.

5.4 Milk composition

There was no significant ($P > 0.05$) effect of *Me* dosing on milk composition when dosed vs. control was compared (Table 5.3). There was however a significant ($P < 0.05$) difference between the butterfat percentage when HCC (3.6%) vs. HCD (2.9%) was compared. The low fat % for HCD group cannot be explained, particularly as the acetic: propionic acid ratios were similar for the HCD and HCC diets. It should be noted, however, that a butterfat depression can be expected when the acetic: propionic acid ratio declines to below 2.2: 1 (Sutton *et al.*, 2003). One possibility is that, although cows were randomly allocated, some of the cows with the lowest genetic potential were placed, by chance, in the HCD group, since cows were not blocked for milk composition.

In the trial of Erasmus (1997), the dosing of cows that received 60% concentrate diet resulted in a significant increase in milk fat percentage ($P < 0.06$). Mutsvangwa *et al.* (2002) reported a milk fat

percentage of 3.03%, a protein percentage of 3.13% and lactose level of 4.85% in a trial conducted on monensin that is used to prevent SARA. Bargo *et al*. (2002) also observed an average milk production of 38.1kg/d and a milk fat of 3.3%. The trial results correspond with these.

The milk fat percentage was not affected by the dosing of *Me* as was expected because there was no significant differences in the acetic acid concentration in the control vs. dosed groups. The percentage of milk fat is primarily influenced by the percentage and digestibility of fibre in the diet (Dugmore, 1995). If dietary fibre in the diet is properly digested, the milk fat percentage will be higher than in the milk of a cow where fibre digestion is depressed.

There was no significant difference (P = 0.82) between the main effects of control vs. dosed on the MUN levels (Table 5.3). There was, however, a significant difference between the MUN levels when LCC (16.33 mg/dl) vs. HCC (13.56 mg/dl) were compared. MUN levels were higher in the low concentrate diet groups. This could be due to the higher CP level and lower energy level in the low concentrate diet compare to the high concentrate diet. It could therefore be speculated that not enough energy were available to utilise enough CP and thus more MUN was then secreted into the milk. The high concentrate diet's CP and energy levels were more balanced and therefore more energy was available to utilise the slightly lower CP levels. Less MUN were therefore secreted into the milk. Acceptable MUN values are between 10 – 16 mg/dl milk (Jonker *et al*., 1998). Erasmus *et al*. (2005) observed an average MUN of 12 – 15 mg/dl in his trial with Holstein dairy cows. Bargo *et al*. (2002) also observed a MUN value of 14.9 mg/dl. A possible explanation for the higher levels of MUN in the low concentrate diets is that there is more energy available in the high concentrate diet for the animal to use to utilize nitrogen in the rumen. Therefore, less nitrogen is excreted in the milk. MUN is normally interpreted as a reflection of the nitrogen to energy balance in the diet. If there is too much protein and / or urea in the diet in comparison to the energy levels, the excessive nitrogen is excreted in the milk, faeces and urine. Nevertheless, although there were differences, the values were within acceptable norms and the differences are, therefore, not biologically important.

Milk protein levels as well as milk lactose percentage were not influenced by the dosage of *Me* (Table 5.3). The average milk protein levels varied between 3.01% and 3.18% which is acceptable for Holsteins producing on average 44 kg milk / day. Erasmus (1997) observed milk protein levels of 3.02 – 3.15% which is similar to our results. The protein percentage of the milk is influenced mainly by the Crude Protein (CP) percentage in the diet, RDP: UDP ratio, amino acid profile of UDP, as well as the amount of microbial protein synthesis. Rumen acidosis can have an effect on the protein percentage in milk. Micro-organisms, such as bacteria in the rumen that digest the feed protein to amino acids and urea, as well as micro-organisms that synthesize protein can be negatively affected by the low pH in the rumen. The lack of any effect of *Me* on milk protein supports the suggestion that the rumen environment and rumen fermentation were not affected to such an extent that microbial protein production was significantly affected.

As expected, there was no effect of dosing *Me* on the lactose levels in the milk (Table 5.3). The average lactose percentage ranged from 4.70 – 4.86%. This correlates with the average levels of lactose in Holstein cows at 4.75% (Holstein Milk recording annual report, 2006).

Table 5.3 The effect of dosing live *Megasphaera elsdenii*, a rumen lactic acid utilising micro-organism, on the percentage of milk protein, milk butterfat, milk urea nitrogen and milk lactose of high producing, early lactation, Holstein cows

	Treatments [1]						Main Effects					
	LCC	LCD	HCC	HCD	S.E.M. [2]	P =	Control	Dosed	P =	Low Conc	High Conc	P =
Milk Protein (%)	3.12^{ab}	3.01^{a}	3.12^{ab}	3.18^{b}	0.05	0.09	3.12	3.09	0.62	3.06	3.15	0.08
Milk Butter Fat (%)	3.34^{ab}	3.58^{a}	3.60^{a}	2.88^{b}	0.24	0.05	3.47	3.23	0.31	3.46	3.24	0.37
Milk Urea Nitrogen (mg/dl)	16.33^{a}	15.44^{ab}	13.56^{b}	14.85^{ab}	0.90	0.23	14.94	15.15	0.82	15.89	14.20	0.07
Milk Lactose (%)	4.70^{a}	4.70^{a}	4.86^{a}	4.81^{a}	0.06	0.71	4.78	4.76	0.75	4.70	4.83	0.03

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

[1] LCC : Low Concentrate Control; LCD : Low Concentrate Dosed; HCC : High Concentrate Control; HCD : High Concentrate Dosed

[2] S.E.M. : Standard Error Mean

5.5. Body weight and Body Condition Score (BCS)

Mean body weight varied between 616 kg and 634 kg and did not differ between treatments (P = 0.99) (Table 5.5). These average weights are in agreement with other studies with similar experimental periods (Erasmus, 1997; Erasmus *et al*., 2005). Body condition score is a helpful tool to evaluate the energy status of dairy cows. The ideal BCS at calving is 3.5 and cows should ideally not lose more than one BCS point (± 60kg) during the first 100 days (Gallo *et al*., 1996). The average BCS for the cows was 2.58 which is in line with recommendations. No treatment differences in body weight or BCS were expected in light of the fact that there were no treatment differences in either milk production or DMI.

5.6. Rumen fermentation parameters

The effect of *Me* dosing on rumen parameters such as pH, VFA and lactic acid is shown in Table 5.4.

5.6.1. Rumen pH

The mean rumen pH was not affected by the dosing of *Me* and the average rumen pH ranged from 5.74 to 5.92. This is similar to the results of the trial conducted by Erasmus *et al*. (2005) where a lucerne maize based diet was fed and an average rumen pH of 5.79 was observed during the first 60 days of lactation. Maekawa *et al*. (2002) also noted an average rumen pH of 5.84 in Holsteins fed a 60% conc.: 40% roughage TMR diet. In an *in vitro* trial that was conducted by Kung *et al*. (1995) it was found that the pH of the rumen culture fluid that was treated with *Me* declined from 6.6 to 5.5 within four hours of fermentation. The pH of the untreated rumen cultures declined from 6.6 to 4.8. This indicates that *Me* has the potential to regulate pH and to prevent a severe drop in pH. Contrary to expectation the average rumen pH was similar between dosed (pH 5.83) and control cows (pH 5.82). A possible reason for the fact that there was no effect on rumen pH could be that, although the lactic acid concentration in the rumen was numerically, but not significant, lower (P = 0.33) (Table 5.4) in the treated animals, especially in the high concentrate diet group, other acidic compounds that are not converted by *Me* to a less acidic substance, could have an effect on the total acid load and on the overall rumen pH. The lactic acid is converted to some of the VFA, for example to butyrate acid (Marounek *et al*., 1989), by *Me* and could then also have a possible effect on the lower rumen pH. Allen (1997) also observed a reduction in rumen

pH when the concentration of VFA increased in the rumen. If the total VFA was less in the treated group, the pH could have possibly been higher as the total VFA can have an affect on the rumen pH.

5.6.2. Rumen Volatile Fatty Acids

There was no significant effect of *Me* on the VFA profile in the rumen (Table 5.4). However, there was a significant ($P < 0.05$) increase in rumen propionic acid in the high concentrate diet compared to the low concentrate diet. This was expected since the high concentrate diet contained more starch and less fibre and the end products of fermentation of these products are more propionic acid and less acetic acid (Sutton *et al*., 2003). Kung *et al*. (1995) observed a decrease ($P < 0.05$) in acetate and propionate when fermentation cultures were inoculated with *Me*. Kung *et al*. (1995) also found that the isobutyrate, butyrate and valeric acid concentrations increased ($P < 0.05$). Others (Marounek *et al*., 1989; Slyter *et al*., 1992) have also reported major accumulations of butyrate when *Me* was grown in pure culture because glucose is fermented to, amongst others, butyrate.

5.6.3. Rumen lactic acid

Lactic acid concentrations were higher in the high concentrate diet compared to the low concentrate diet ($P < 0.01$) primarily due to the higher starch content and lower fibre content of the high concentrate diet. Although the lactic acid concentration in dosed cows was numerically lower, it was not statistically different ($P > 0.05$). Kung *et al*. (1995) found a significant ($P < 0.05$) reduction in lactic acid (D and L-lactate) after the rumen fluid culture was treated with *Me* compared to the control culture. This could indicate that *Me* could be used to reduce the lactic acid concentrations in the rumen and, therefore, assist in the prevention of rumen acidosis. As mentioned previously, some dietary and management factors probably contributed to a lack of treatment response on lactic acid concentration.

Table 5.4 The effect of dosing live *Megasphaera elsdenii*, a rumen lactic acid utilising micro-organism, on rumen fluid pH, rumen fluid VFA and lactic acid concentrations of high producing, early lactation, Holstein cows

	Treatments [1]						Main Effects					
	LCC	LCD	HCC	HCD	S.E.M. [2]	P =	Control	Dosed	P =	Low Conc	High Conc	P =
Average pH	5.85	5.92	5.78	5.74	0.11	0.63	5.82	5.83	0.91	5.88	5.75	0.28
Acetic acid (mmol/100ml)	7.41	7.58	7.89	7.45	0.40	0.46	7.65	7.52	0.74	7.49	7.67	0.65
Propionic acid (mmol/100ml)	3.38^{a}	3.67^{ab}	4.26^{b}	4.19^{ab}	0.31	0.56	3.81	3.93	0.71	3.51	4.22	0.03
Butyric acid (mmol/100ml)	0.93	0.95	1.02	0.84	0.07	0.18	0.97	0.89	0.27	0.94	0.93	0.88
Iso Butyric acid (mmol/100ml)	0.08	0.08	0.08	0.07	0.01	0.63	0.08	0.08	0.43	0.08	0.08	0.79
Valeric acid (mmol/100ml)	0.15^{a}	0.17^{ab}	0.25^{b}	0.23^{ab}	0.03	0.52	0.20	0.20	0.96	0.16	0.24	0.02
Acetic acid : Propionic acid ratio	1.80^{a}	1.75^{ab}	1.54^{b}	1.52^{b}	0.09	0.85	1.67	1.63	0.64	1.77	1.53	0.01
Lactic acid (mg/100ml)	2.75^{a}	2.50^{a}	5.80^{b}	4.32^{ab}	0.88	0.48	4.28	3.41	0.33	2.63	5.06	0.01
Total VFA (mmol/100ml)	11.93	11.64	11.41	12.85	1.09	0.43	11.67	12.24	0.60	11.78	12.13	0.75

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

[1] LCC : Low Concentrate Control; LCD : Low Concentrate Dosed; HCC : High Concentrate Control; HCD : High Concentrate Dosed

[2] S.E.M. : Standard Error Mean

5.7 Faecal samples

Faecal score was not affected by the dosage of *Me* with the average faecal score for the control diet being 2.64 and for the treated group 2.58 (Table 5.5). The ideal faecal score for a high producing cow on a high concentrate diet is between 2 and 3 (Hall, 1999) which is in the same order as these results. The high concentrate diet clearly did not disrupt rumen fermentation to such an extent that SARA developed and faeces become wet and slushy which is typical of acidosis.

There was a significant difference ($P = 0.016$) between the dosed and control animals in faecal pH. The faecal pH of the dosed animals (6.62) was higher than the control animals (6.43) and thus less acidic. This suggests that less starch may have been digested in the hindgut of cows in the treated groups, possibly due to more starch being digested in the rumen (Erasmus, 1997). This in turn suggests that the addition of *Me* may have had a beneficial effect in the rumen, e.g. preventing lactic acid accumulation and its detrimental effect on rumen fermentation in general. This is supported by the faecal starch content data (Table 5.5).

Table 5.5 The effect of dosing live *Megasphaera elsdenii,* a rumen lactic acid utilising micro-organism, on the body mass, body condition score and faeces pH, faeces score and % starch in the faeces of high producing, early lactation, Holstein cows

	Treatments [1]						Main Effects					
	LCC	LCD	HCC	HCD	S.E.M. [2]	P =	Control	Dosed	P =	Low Conc	High Conc	P =
Average Mass of cows (kg)	634	616	634	616	10.47	0.99	634	616	0.09	625	625	0.98
Body Condition Score (BCS) (1 - 5)	2.55	2.61	2.59	2.59	0.15	0.77	2.57	2.6	0.76	2.58	2.59	0.89
Faeces pH	6.47^{a}	6.72^{b}	6.39^{a}	6.53^{ab}	0.08	0.49	6.43^{a}	6.62^{b}	0.02	6.59	6.46	0.09
Faeces Score (1 - 5)	2.63	2.46	2.66	2.70	0.09	0.24	2.64	2.58	0.50	2.55	2.68	0.16
Faeces Starch (% DM)	3.98^{a}	2.43^{b}	4.34^{a}	3.81^{ab}	0.53	0.35	4.16^{a}	3.12^{b}	0.06	3.21	4.08	0.11

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

[1] LCC : Low Concentrate Control; LCD : Low Concentrate Dosed; HCC : High Concentrate Control; HCD : High Concentrate Dosed

[2] S.E.M. : Standard Error Mean

The faecal starch content was also affected (P = 0.055) by *Me* dosage (Table 5.5). The treated animals had a lower starch content (3.12%) in the faeces compared to the control animals (4.16%). This could indicate a possibly better starch digestion in the rumen because less starch had flowed through the rumen and small intestine to the hindgut and excreted in the faeces and more starch was digested in the rumen (Table 5.6) (McDonald *et al*., 2002). The starch % in the feed and faeces is illustrated in Table 5.6.

Table 5.6 The effect of *Megasphaera elsdenii,* a rumen lactic acid utilising micro-organism, on the starch utilization (% starch in feed vs. % starch in faeces) of early lactation high producing Holstein cows

	% starch in feed	% starch in faeces
LCC	25	3.98
LCD	25	2.43
HCC	30	4.34
HCD	30	3.81

LCC : Low Concentrate Control
LCD : Low Concentrate Dosed
HCC : High Concentrate Control
HCD : High Concentrate Dosed

Theurer (1986) demonstrated that changes in ruminal starch digestion may affect the amount of starch that flows to the small intestine. All this starch may not always be digested in the small intestine and it is possible with high starch diets that decreased starch digestion in the rumen may ultimately be reflected in increased faecal starch (Theurer, 1986; Dunlop, 1972). It is postulated that the reduced faecal starch content observed for dosed cows in this study indicated some improvement in ruminal starch digestion, therefore suggesting a more favourable rumen environment.

Increased starch flow to the small intestines may result in increasing quantities flowing to the large intestine where digestive efficiency is inefficient (Harmon & McLeod, 2001). Harmon *et al*. (2004) found a decline in starch digestibility in the small intestines with an increase in the amount of starch entering the small intestine. From the above mentioned results, it can be concluded that, if too little of the starch is fermented in the rumen due to poor rumen health and un-balanced micro-organism population, too much starch flows through to the small intestines so that even the enzymes in the small intestines do not have the capacity to digest all the starch and the undigested starch flows through to the large intestines (Harmon & McLeod, 2001).

5.8 Health

The health status of cows was monitored on a regular basis. When the DMI and/or milk production declined by more than 30 – 50% compared to the previous week's DMI and/or milk production for five days or more due to any type of illness, the cow was culled from the trial. The cow was than replaced with a healthy cow that had recently calved. The replacement cow had the same milk production potential, based on the previous lactation, as the replaced cow. The replacements were necessary to determine the effect of the *Me* dosing on the production of healthy cows. Eleven cows were culled from the control group and only two from the treated group (Table 5.7). All the culled cows were replaced, thus 15 cows per treatment completed the trial. Only the data from the cows that completed the trial were used.

Data from Table 5.8 support the argument that the lack of response, based on the criteria set for culling and replacing animals, was not due to specific health problems, but to the general stress of high production levels. It is clear that most of the health problems experienced by cows that were later replaced, were also experienced by cows that successfully completed the trial.

Results therefore suggested that, with *Me* dosing, the percentage of concentrates in the diet could potentially be increased without the negative effect of a decreased DMI and/or milk production for periods of five days or more. This could indicate that the dosage of *Me* could have a positive effect on the health of the rumen as on well as the whole animal.

The culling aspect should be carefully considered when designing trials since it can have a significant effect on the results. A different approach could have been taken by only culling the cows with non-nutritional disorders. The reason is that this trial is a nutrition related trial, and nutritional disorders should be part of the health data. The question remains as to what the outcome would have been if all animals remained in the trial.

Table 5.7 The effect of *Megasphaera elsdenii,* a rumen lactic acid utilising micro-organism, on the number of high producing, early lactation Holstein cows culled from the trial due to a decrease in DMI and/or milk production of more than 30 – 50% for five days or more, compared to the previous week's DMI and/or milk production

Number of cows culled from trial due to continuous illness					
LCC	LCD	HCC	HCD	Control	Dosed
4	1	7	1	11	2

LCC : Low Concentrate Control
LCD : Low Concentrate Dosed
HCC : High Concentrate Control
HCD : High Concentrate Dosed

Table 5.8 The number of incidences of different animal health problems of the original plus replacement cows that completed the study (Compl) and number of original cows that were replaced (Repl), that were diagnosed at some point in the trial

	LCC		LCD		HCC		HCD	
Health problem	Compl	Repl	Compl	Repl	Compl	Repl	Compl	Repl
Endometritis	4	2	2	1	2	3	6	1
Fever	2	2	2			2	1	1
Hoof problems	2		1			1		
Mastitis	1	1	2	1	1	1	1	1
Oedeme		1	1		1		1	
Diarrhoea						1	1	
Bloat	1		1					
Septicaemia							1	
Anaplasmosis	1							
Dehydration	1							
Anaemia			1					
Left displaced abomasum		1				1		
Abscess		1						
Internal inflammation		1			1			1
Peritonitis						1		

LCC : Low Concentrate Control; LCD : Low Concentrate Dosed; HCC : High Concentrate Control; HCD : High Concentrate Dosed

Chapter 6

Conclusion and recommendations

This trial was conducted to determine the effect of live *Megasphaera elsdenii,* an anaerobic rumen micro-organism, on the productivity and health of Holstein dairy cows fed TMR's with either 60% or 70% concentrate.

In general the results indicate no clear advantage of dosing cows with *Me* for either concentrate levels. Primary production parameters such as milk production and feed efficiency as well as rumen parameters (VFA, pH, and lactic acid), were not affected by dosing. However, faecal starch and faecal pH were significantly influenced by dosing in a manner which suggests that *Me* dosing may have a positive effect on total tract starch digestion. Furthermore *Me* dosing reduced the number of animals that were culled from the trial due to severe intake depression as a result of illness.

Another factor that may have confounded the outcome of the study was the removal of cows with serious intake and/or production problems, as a result of illness, from the trial. Eleven of the original 30 cows from the control group were culled, as compared with only two of the original 30 cows from the treatment group. It may be reasoned that, if no cows were culled from the trial due to poor production, the control cows would have performed worse than the *Me*-treated cows. This would have been more pronounced on the 70% concentrate diet where rumen acidosis would be expected to be a greater problem.

Rumen acidosis is an economically significantly problem for high producing dairy cows. This novel DFM *Me* could hold promise as a possible solution to assist in the prevention of rumen acidosis as has been suggested by this and other trials. Unfortunately there is still a scarcity of supporting data which indicates that it is important for further trials.

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