The effects of *Megasphaera elsdenii* on dairy heifer performance

by

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DECLARATION

I, Lenkie Magapu Dikotope declare that this dissertation titled "***The effects of Megasphaera elsdenii dairy heifer performance***", is my own work. It is being submitted for the Master of Science in Agriculture at the University of South Africa, Pretoria. This dissertation was not submitted earlier for any degree in any other academic institution.

Name: Lenkie Magapu Dikotope
Date: December 2018
Signature:

DEDICATION

This qualification is dedicated to my late father, John Kgagudi Dikotope, he was an advocate for education. His wish had always been to afford his children the gift of education.

I wish to carry on his legacy onto my son, Boikanyo Justin Dikotope, I hope he finds this inspiring and follow in my footstep and find hope and treasure in education.

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ABSTRACT

The aim of this study was to evaluate the effects of *M. elsdenii* (*Me)* dosing on dairy heifer performance. A secondary set of data (feed intake, heifers birth weights, age and Weight at insemination, and first lactation milk performance) of heifers (dosed and not dosed with *Me*) was obtained from the dairy herd of the Agricultural Research Council – Animal Production. Data were arranged in a complete randomised design and analysed as repeated measures. Milk, pre-weaning starter and metabolised energy intake did not differ between the control and the *Me* groups. Post-weaning starter feed intake was higher (p=0.03) for *Me* fed heifers than control heifers. The post-weaning metabolisable energy intake was also higher (p=0.03) for heifer fed *Me* than control heifers. The average daily weight gain of heifers dosed with *Me* was higher during the pre-weaning period (0.66 kg/day; p=0.04) and after weaning (1.12 kg/day; p=0.03) compared to control (0.60 and 0.65 kg/day, respectively). At 42 and 70 days old, the BW of *Me*-heifers was greater (75.8 ± 2.6 and 91.2 ± 4.6 kg) than control heifers (61.9 ± 2.6 and 77.2 ± 4.6 kg) ($p<0.05$). There was no difference ($P>0.05$) in BW at insemination, number of insemination and milk yield between the two groups of cows ($p>0.05$). Early feeding of *Me* to heifers in the present study positively affect heifer growth during and early after milk feeding period, confirming previous report. Animal weight at puberty and the subsequent milk production were not influenced by feeding *Me*. It is possible that *Me* did not survive long after weaning to continue to express its influence on animal performance.

Keywords: Heifer, direct fed microbial, dairy calves, mature cows

Table of contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADG: Average daily gain
ARC: Agricultural Research Council
BW: Body Weight
Co: Cobalt
Cu: Copper
DFM: Direct Fed Microbial
DCP: Dicalcium phosphate
DMI: Dry Matter Intake
DNA: Deoxyribonucleic Acid
Fe: Iron
FWI: Free Water Intake
GIT: Gastro Intestinal Tract
hrs: Hours
IgA; Immunoglobulin A
IgG: Immunoglobulin G
IgM: Immunoglobulin M
I: Iodine
kg: Kilogram
L: litre
Me: *Megasphaera elsdenii*
ME: Metabolizable Energy
Mn: Manganese
MR: Milk Replacers
N: Nitrogen
NDF: Neutral Detergent Fiber
RDP: Rumen Degradable Protein
RUP: Rumen Undegradable Protein
Se: Selenium
VFA: Volatile Fatty Acids

CHAPTER 1
INTRODUCTION

The goal of dairy farms is to produce the best cows from replacement heifers. Raising replacement heifers successfully is not only important for the future herd, but it is also important financially. Calf management and feeding system influence growth performance through intake of starter feed as well as health (Uys *et al*., 2011). Previous studies have demonstrated that dairy heifer calves that achieve greater weaning body weight (BW) and increased growth rates from birth to weaning is reported to be associated with earlier attainment of puberty (Macdonald *et al*., 2007). Rapid rumen development before weaning is important because it affects health, growth rates and intake of solid feeds after weaning (Muya *et al*., 2015). The calf's rumen needs to develop quickly for the calf to digest solid feeds and minimise dependency on milk.

Macdonald et *al*. (2007) indicated that the BW influence more attainment of dairy heifer's puberty than the age. It is also known that the nutritional status between calf birth and puberty can cause long-lasting effects on the capability of adult cows to produce milk. Drackley (2005) compared calves fed restricted quantities of milk replacer with calves fed *ad libitum* milk, before weaning. Calves that suckled had greater BW improvements and milk production increased in the first lactation due to greater nutrient intake pre-weaning. Other studies (Moallem et *al*., 2010; Soberon et *al*., 2012; Lohakare et *al*., 2012) have shown that increase in nutrient supply in new born dairy calves possibly improved first and probably successive milk production. In the study of Soberon et *al*. (2012), the pre-weaning average daily gain (ADG) of calves accounted for 22% of the variation in first lactation milk yield and more milk was produced by animals that had greater nutrient intake from milk or milk replacer during the pre-weaning period. In studies by Zanton et *al.* (2005 & 2007) growth rate of almost 800 g/day from 150 to 320 kg BW was prerequisite to maximise first milk lactation and protein yields in eight studies when calving BW was included in the model.

The feeding of direct fed microbial (DFM) to neonatal dairy calves has proven to be a suitable feeding management tool to improve calf performance. In a recent study (Muya et *al*, 2015) feeding *Megasphaera elsdenii* (*Me*), predominant lactate-utilizing bacteria, enhanced the growth performance through improved feed intake. Furthermore, the increased nutrients intake resulted from greater epithelium metabolism and enhanced absorption of digestive end products. *Megasphaera elsdenii* has potential to increase ruminal butyrate production. Butyrate stimulates the development of papillae and increases the ability of solid feed intake (Muya et *al*., 2015). The objective of implementing strategies to improve performance of dairy calves is to obtain good replacement heifers for future milk production. Although supplementing neo-natal dairy calves with Me as shown improved DMI and growth during early age, no study has demonstrated its effects on heifer's reproduction and cow's lactation performance. It is possible that increased starter feed intake and growth soon after Me supplementation can lead to improved heifer growth, conception and sub-sequent lactation.

It is hypothesized that improved calf performance with Me supplementation would lead to enhanced heifer maturity, desired critical mating weight and improved first lactation performance. Thus, the objectives of this study are:

- To determine the effects of dosing *Me* during the pre-weaning period on BW and age at first insemination
- To ascertain the effects of dosing *Me* during the pre-weaning period on milk yield and composition during the first lactation
- To determine the correlation between the pre-weaning ADG and weaning BW with milk yield during the first lactation on calf BW as affected by *Me*

CHAPTER 2
LITERATURE REVIEW

2.1. Feeding the dairy calves

The most sensitive nurturing period for calves is from birth until the age of three months. The success of this nurturing period, besides biological, environmental and nutritional stress depends mostly on calf managers paying special attention to calf nutrition program (Miller, 2012). Establishing a quality nutrition for young calves is crucial. Proper feeding management is the most important aspect of a calf's life. Calves are born with little to no antibodies, and an immature immune system (Cabral, 2014). A fully developed calf's immune system is not properly developed until the age of eight months. Therefore, lack of immune system in calves makes them vulnerable to pathogens and diseases.

2.1.1. Liquid feeding

Dairy producers use various liquid feeds for early calve feeding. These feeds include milk, waste milk, whole milk, transition milk, colostrum, and milk replacers (Uys, 2008; Godden, 2008). All these feeds provide excellent results in-terms of the growth of dairy calves, however, choosing to use them largely depends on financial situation and accessibility. Saleable milk is less valuable when fed to calves than when sold (Uys, 2008). The dairy producers will either feed young calves' non-saleable milk or milk replacer instead. In South Africa, various milk producers utilize a range of all non-saleable milk (colostrum, transition milk, and milk withheld after drug treatment) for calve feeding (Uys, 2008).

Although whole milk is considered to be an exceptional nutritional feed, the risks associated with consumption of possible pathogenic organisms in unpasteurised waste milk are well known (Selim & Cullor, 1997). On-farm pasteurisation revealed that the growth and health of calves under field conditions were suitable (Godden et *al*., 2005). The calves fed conventional milk replacer had substantially less body gain and were more vulnerable to disease than calves fed pasteurised non-saleable milk (Godden et *al*., 2005). The challenge of feeding waste milk is the likelihood of

antibiotics presence (Uys, 2008). Milk contaminated with antibiotics might change gut microflora of calves and cause digestive disorders.

A study by Selim & Cullor (1997) reported that *Escherichia coli* cultured from waste milk samples showed resilient to antibiotics with less than one third of the samples being sensitive to tetracycline or ampicillin. Wray (1990) reported that the milk comprising antibiotics is indigestible and calves fed milk with antibiotics had reduced daily live weight gain High-quality milk replacers are exceptional for young calves because they are cheap and have minimal risks. Milk replacers are consistent product for daily usage, easy to store, and to control disease. Keeping such uniformity in the diet for young calves is vital, as to minimize chances for digestive upsets (Uys, 2008). The importance of this consistency maybe useful when young calves are reared under stressed environment, such as cold or wet weather or during disease outbreaks (Uys, 2008).

Milk replacer nutrient content needs to correspond to the preferred amount of calf growth (Uys, 2008). Crude protein (CP) content of 20% to 22% increases lean tissue growth when calves are on conventional restricted feeding programs (Bartlett et *al*., 2006). For calves on more aggressive liquid feeding programs intended to improve early growth rates, CP must be in the range of 26% to 28% (NRC, 2001; Van Amburgh & Drackley, 2005). The critical dynamics of protein are digestibility, amino acids content, amino acid balance and the presence of anti-nutritional factors (Gilani et *al*., 2005). Fat is the main variable caused by the variance in energy content of milk replacers (Uys, 2008). The daily gain increase depends on the fat content increase, this might result in reduction of starter intake (Kuehn et *al*., 1994). The lower fat content of milk replacer during thermoneutral conditions, intent to support lean tissue growth and increase in starter intake. During cold feeding period, greater fat content is required (Drackley, 2008).

2.1.1.1. Colostrum

Calves are born agammaglobulinemic, meaning they are born with little to no antibodies, and an immature immune system (Cabral, 2014). To develop full immune system, calves must obtain antibodies which they can ingest through colostrum, and

then absorb the immunoglobulins (Ig) across the small intestine. Colostrum has different quantities of carbohydrates, fats, proteins, peptides, minerals, vitamins, non-peptide hormones, cytokines, enzymes, polyamines, and nucleotides in different amount (Hadorn, 1997). The Ig found in colostrum contains proteins. The absorptions of these proteins occur in the epithelial cells that line the gastrointestinal tract, called pinocytosis (Cabral, 2014). The process of absorbing Ig is referred to as passive transfer. Passive transfer occurs in the first four (4) hours post-partum and gradually declines till it stops after 24 hours. Therefore, feeding calves with colostrum throughout the first 24 hours is ideal to ensure they receive as many Ig as their gut can absorb. Flower, (1999) showed that with effective passive transfer, a better growth rate, and lower mortality are observed compared to calves with failed passive transfer.

Colostrum contains many elements that are beneficial to the health of the neonatal calves, including but not limited to providing vital antibodies and providing important amounts of non-Ig immune factors such as leucocytes and cytokines (Queigley, 1997). Immunoglobulin plays an important part in the development of the immune system of a calf (Conneely et *al*., 2013). There are three types of immunoglobulins such as immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA), which account roughly 80-90%, 5%, and 7%, respectively (Godden, 2008). There is a link between these antibodies and with allergic reactions, when the immune system reacts excessively due to environmental antigens (pollen, fungi and spores and is found in the lungs, skin and mucous membranes).

The concentration of IgG is considered to be hallmark of evaluating the quality of colostrum. The first benchmark is the microbiological quality as determined by the role of low bacterial contamination, and nutrients are considered the second benchmark and important element of colostrum (Santos et *al.,* 2017; Conneely et al., 2013; Cabral, 2014). High levels of crude fat and casein found in colostrum increase the energy content which is critical in thermogenesis or body heating (Cabral, 2014). The presence of cytokines and growth factors in colostrum are important, but they not completely understood. Bacteria are also present in colostrum, which leads to contamination and potential blockage of immunoglobulin absorption (Schoombee, 2015). In order to minimise the number of bacteria in colostrum and increase its

quality, is to pasteurize the colostrum, this is recommended for intensive milk replacer feeding program/ pasteurized milk feeding program. Godden (2008) stated that the best pasteurization of colostrum is achieved at low temperature and over a long-time approach at 60^{o}C for 60 minutes.

In systems where colostrum is fed, if the harvested colostrum is not used right away, it is best to store it for future use. Storing colostrum in cold environments proved to have an impact in decreasing the survival of pathogens and the possibility of pathogenic incubation (Stamey, 2006). To preserve the high quality of harvested colostrum it should either be frozen (0^{o}C) or refrigerated (5-7^{o}C) for future use. Colostrum stored in a clean, covered container can be cooled for a week and frozen for a year unless it is reheated (Schoombee, 2015). However, the refrigerated colostrum has a potential of developing high concentration of bacteria within the first two days. Therefore, it is best to freeze colostrum to minimize the bacteria counts (Cabral, 2014). Temperature control is vital when thawing frozen colostrum, heating temperatures should be kept at 60^{o}C to avoid overheating of colostrum. Should temperatures be greater than 60^{o}C, denaturing of the immunoglobulins can occur resulting to a decrease in optimal quality and absorption of antibodies (Godden, 2008).

If 50 mg/ml of immunoglobulins is present in a colostrum, that colostrum is of high quality and best for calf's feeding. To measure its quality (colostrum), it is best to use a colostrometer as cow-side colostrum testing method. The colostrometer uses gravity to estimate the IgG and solid concentration in the colostrum (Cabral, 2014). The test can take up few minutes, to differentiate between high and low quality of colostrum. The colostrum readings are temperature depended, so it advisable to conduct tests at room temperature (23^{o}C) to obtain consistent and accurate readings (Godden, 2008).

If the test shows that the colostrum is of low quality, colostrum should be thrown away. For example, if colostrum is bloody or has a tint of pink, it shows high presence of red blood cells and it should be thrown away. If colostrum with high red blood cells is fed to calves, then it has a potential of causing diarrhoea due to its makeup of gram-negative bacteria (Lorenz et *al*., 2011).

The calf survival is influenced by the suitable feeding routine of high-quality colostrum. During the calving process, the immune system of the calf suffers from the release of corticosteroids therefore, it is essential for the calf to ingest colostrum of great quality immediately (Lorenz et *al*., 2011). Approximately 10-12% of the calf's birth weight is used to determine how much high-quality colostrum a calf should be fed. For example, a 41 kg calf would be fed 4L of colostrum at zero hours (Cabral, 2014). At 12 and 24 hours, calves should be fed 2L of colostrum for a total of 8L of colostrum in the first 24 hours (Cabral, 2014). The recommended method of feeding calves is hand feeding instead of letting calves suckle from the dam. A high percentage of failure passive transfer are noticed when calves were left to suckle from the dam (Stamey, 2006). Hand feeding helps to control the exact amount of colostrum a calf should consume. Since it is known how much colostrum a calf should be fed, it is best to use nipple bottle or esophageal feeder. Feeding using esophageal tube is a faster way to feed calves, but the use of esophageal feeding should be minimized because it can lead to an upset stomach and improper absorption of nutrients.

2.1.1.2. Whole milk

When feeding whole milk, it is recommended to divide the milk into portions and feeding should take place during the first seven days at (3-4) equal intervals (Hashmi, 1997). If whole milk will be replaced with skim milk this can be done at a gradual pace. The presence of enormous quantity of foam is unacceptable in the skim milk. The foam should be removed by using pad or small card board (Hasmi, 1997).

It is common practice to feed whole milk for the first two weeks of life at 10% of birth weight every day (Amaral-Phillips et *al*., 2006). To minimize the feeding cost, waste milk and excess colostrum can be used as alternative feeds (Heinrichs et *al*., 1994). All feeding utensils, buckets, and bottles should be thoroughly cleaned and sanitized to control the spread of diseases.

2.1.1.3. Milk replacer

Milk replacers (MR) are exceptional, convenient and well formulated to feed young calves. They are in a form of powder and is mixed with water prior to feeding (Uys, 2008). MR are less expensive per unit of nutrient supplied than whole saleable milk and are made from by-products of the milk manufacturing industry. Major factors such as economy, convenience and biosecurity make MR a preferred liquid feed to dairy calf farmers.

2.1.1.4. Drinking water

Water is vital for all living animals and cattle meet this requirement through drinking water. Calves have access to water during liquid feeding stage through milk or milk replacer (Yavuzi, 2015). For good husbandry, enough fresh, clean water must be provided to calves.

Water is the most critical nutrient in liquid feed. It plays an essential role in numerous processes such as the regulation of body temperature, growth, digestion and metabolism. c. So it is crucial that calves have access to unrestricted water in addition to their liquid intake. The calf's life is affected by the amount of water intake (Cabral, 2014). For example, the amount of starter intake depends on calf's water consumption. Water is essential for the majority of life's developments, for example transportation of nutrients and helps in the digestion and metabolism of nutrients; disposal of waste materials (urine, faeces, and respiration) and excess heat (perspiration) from the body; preservation of suited fluid and particle stability in the body; and establishment of a fluid environment for foetus development (Murphy, 1992). Bartlett et *al.,* 2006, suggested that in young calves the empty body tissues, the component that is deposited in large quantities during growth is made up of approximately 75% water. The starter intake is depended on water consumption (Kertz et *al*., 1984). Calves that drinks enough water do not experience scours, however, calves that experience scours have a tendency of increasing their water consumption if it is freely accessible (Kertz et *al*., 1984). Young calves should always have water at their disposal. During cold weather young calves should be given small quantity of warm water after feeding and at noon (Uys, 2008). Separation of

water and dry feed containers is important, because it stops water spill into the dry feed.

After weaning calves drink approximately 10-15L/day and up to 25L/day during hot days (Moran, 2012). Moran, (2012) indicated that it is essential for dairy calves to drink 4L of water for every kg of concentrate they consume in order to achieve optimal feed efficiency. Calves need constant access of fresh water, the moment they begin consuming solid feeds, especially dry feeds such as hay or straw (Ghassemi, 2012). This will improve solid feed intake and reduce weaning age (Murphy, 1992). Research showed that water consumption increases by 0.05 kg/day for every gram of sodium intake, and diets high in salt sodium bicarbonate, or protein also fuel water consumption.

2.1.2. Solid (starter) feeding

There is variety of calf starters, numerous features add significant changes in calf starter consumption (Chester-Jones et *al*., 2009). High-quality calf starter should contain 20-24% crude protein (Hashmi, 1997) and 80 percent total digestible energy. The amount of starter intake, as well as protein percentage and fat found in milk replacer, gets affected during milk feeding program (Yavuzi, 2015). Water is one of nutrient for the calf and its availability has significant impact on starter intake.

Calf starter consumption is influenced by factors such as formulations and physical form of the starter, calf birth weight, calf genetic make-up, and gender (Chester-Jones et *al*., 2009). Furthermore, environment, management, and housing have large impact on how well starter feed is consumed by calf (Yavuzi, 2015).

Calf starter consists of sufficient combination of grains, protein source, minerals and vitamins. Table 2.1 shows calf starter nutrient content composition and many different ingredients that can be used. It is crucial that young calves start early to consume starter to encourage rapid rumen development and to allow early weaning (Davis, 1998; Soberon, 2012). In addition, it helps in reducing feed costs and management time (Kertz, 1984). Rumen starts to develop when the calf starts eating calf starter and drinks water. At the age of four days, calves should be fed calf

starter. Research indicates that feeding calves water increases starter intake and weight gain (Ghasseni et *al*., 2012).

The calf starter's structure influences palatability and consumption. There are different structural arrangements such as coarse-textured, pelleted or in a rolled meal (Chester-Jones et *al*., 2019). When too hard and too soft pellet are used, they will affect calf consumption. Furthermore, calves do not prefer finely powdered starters (Davis, 1998). There are contradicting results from several experiments on intake and performance of calves receiving starters in different physical forms (Owens et *al*., 1997; Franklin et *al*., 2003). Ghassemi et *al*., (2012)., reported higher intake and lower gain when ground starters were replaced with pelleted or textured starter. However, Porter, (2007) found that calves consumed more coarsely ground than pelleted starter. Franklin et *al*., (2003) reported greater consumption and average daily increase on calves fed coarse starter compared to those fed pelleted starter.

Calf starter should have a minimum level of roughage by-products. Calf starters comprising of 5-8% molasses or molasses-based mixture products advances palatability and consumption (Yavuzi, 2015). A study conducted at Penn State University, observed coarse calf starter with 5% molasses content against those with 12% molasses over 42 days period. The starter sugar content was considerably increased within the starter containing additional molasses. A significant decrease in post weaning and overall starter DM intake, overall total DM intake, daily heart girth change, and final heart girth was observed, however a tendency to decrease the overall daily gain when calves were fed starter having 12% molasses was observed. Though, there was a considerable increase in the concentration of blood volatile fatty acid once calves receive a starter containing 12% molasses. No major variance was observed among calves fed starter containing 5 or 12% (Lesmeister, 2005).

The average daily gain target from birth through weaning is 0.80 kg/day with a dry matter intake of 0.91 kg/day (Uys et *al*., 2011). A balance in energy and crude protein ratio is essential in order to meet the calf's need and to achieve the set growth goals. Calf starter should contain 20-24% crude protein (Hashmi, 1997). Table 2.1 demonstrates different calf starter ingredients with its composition

percentage; these are the proposed and precise requirements of calf starter feed. The mineral requirements should meet with the following mixture of salts as recommended on (Table 2.2), for calf starter (Hasmi, 1997).

Table 2.1: Composition of calf-starter (Hasmi, 1997).

Ingredients	Composition (%)
Skimmed milk powder	30
Crushed (barley /maize/wheat/rice broken/rice polishing (Last step)	30
Protein concentrate of groundnut cake/cotton seed cake (decort)/soybean meal (whole)/Til cake	32
Mineral mixture (Table 2.2 below	2.6
Molasses	5.0
Salt	0.5
Antibiotics (aurofec/terramycin)	0.1
Antioxidant butylated hydroxy toluene/butylated hydroxy anisole/thoxyquin/carotenoids/Vit. E.	0.1

Table 2.2. Mineral mixture for calf starter (Hasmi, 1997).

Mineral in feed	Quantity in feed	Name of salt	Concentration (%)
Calcium (Ca)	0.77 (%)	Dicalcium phosphate (DCP)	1.7
Phosphorus (P)	0.59 (%)	DCP	1.7
Magnesium	0.10 (%)	Magnesium carbonate	0.1
Sodium (Na)	0.06 (%)	Sodium chloride (NaCl)	0.5
Potassium (K)	0.80 (%)	Potassium chloride (KCl)	0.8
Manganese	10 mg/kg	Manganese sulphate ($MnSO_4$)	0.003
Zinc (Zn)	20 mg/kg	Zinc carbonate ($ZnCO_3$)	0.020
Iron (Fe)	10 mg/kg	Ferrous sulphate	0.025
Cobalt (Co)	0.1 mg/kg	Cobalt acetate	0.003
Iodine (I)	0.08 mg/kg	Potassium iodide (KI)	0.003
Copper (Cu)	4.00 mg/kg	Copper sulphate	0.009
Selenium (Se)	0.1 mg/kg	Selenium sulphide (SeS)	0.007

2.2. Calf growth and nutrient requirements

2.2.1. Dry matter intake

Dry matter intake (DMI) is vital in calf's nutrition, as it determines amount of nutrition available to an animal for health and production (Murphy, 1992). Dry matter intake is directly related to live weight (Santos et *al*., 2017). Underfeeding and overfeeding can be prevented by making an accurate estimation of DMI when formulating diets and by doing so you will be promoting the efficiency of nutrient use. Feeding less nutrients restricts production and negatively impacts animal health while feeding more nutrients escalates feed expenditure (Murphy, 1992). Additional nutrients may cause deposition in an area that might be poisonous or cause health effects if the amount is high.

Low digested feedstuff intends to place restriction on DMI as a result of speedy movement from the rumen and passage through the digestive tract. The reticulorumen and abomasum receptors, which has elastic and touchy walls results in undesirable effect on DMI as digestive weight and volume of digestive accumulates (Queigley and Drewry, 1998). The high neutral detergent fibre (NDF) fraction, is a vital dietary component linked to the fill effect due to its low digestive rate (Yavuzi, 2015).

2.2.2. Energy

A portion of total (gross) energy consumed by calves is available after digestion and is vital for sustainable body temperature and to support normal body functions.
Ruminant's maximum undigested energy is lost through faeces, while minimum quantity of the digested energy is lost through rumen fermentation and in urine (Soriano, 1998). The valuable energy remaining after energy is lost through faeces, urine, and combustible gas substrate is referred to metabolizable energy (ME) (Soriano, 1998). The metabolizable energy consists of more than 90% of the gross energy obtained in milk, compared to hay and concentrates with only makes 50-60% of the gross energy (Soriano, 1998). Age and live weight determine the amount of energy required for growth. Energy content of the feed plays an important role.

Medium quality pasture or hay is not often used for growth performance compared to high energy feed such as milk and concentrates (Soriano, 1998).

2.2.3. Protein

Calves require proteins daily to maintain biological processes, tissue repairs and blood formation (Tadele and Getachew, 2015). Proteins are also vital in calve's growth, such as the laying down of muscles. It is important to feed calves with balanced diet containing all the essential amino acids. Protein synthesis takes place in many organs, including liver and skeletal muscle, which is stimulated by insulin, so the increase in insulin following a meal also promotes protein synthesis during this period (Frandson et *al*., 2009).

Building blocks of all proteins are amino acids. Amino acids are important for maintaining, growth, reproduction, and lactation of dairy cattle (Moran, 2012; Tadele and Getachew, 2015). Livestock needs more than 20 specific amino acids. Calves absorb and resynthesize feed protein broken down into amino acids through digestion for maintenance and growth (Tadele and Getachew, 2015). Rumen microbes produces various amino acids in older calves, and other amino acids are provided by undegraded digestible dietary protein. Amino acids play an essential role in forming proteins and when in excess can be converted to energy. When formulating rations, it is critical not to exceed the required amount because protein is generally not cheaper than energy supply in feeds (Moran, 2012).

The nitrogen (N) element is an essential component of all proteins consisting of about 16% (Moran, 2012). It is assumed that feed has average N content of 16 g in every 100 g of protein (Soriano, 1998). When feeding the crude protein in calves up to 6 months it should be mainly in the form of true protein (Moran, 2012). This will improve its efficiency in calf by supplying required amino acids. It is less likely that any amino acids can set restrictions to calf's peformance or any surplus amino acids being lost as protein sources (NRC, 2001).

Fish meal is an animal protein which is important to calves than plant proteins, since its amino acid component is similar to that of a fast growing calf (Moran, 2012). The use of animal protein to feed ruminants has been banned in most countries due to concerns related to spread of mad cow diseases (Lee et *al*., 2013).

Rumen degradable protein (RDP) and undegradable dietary protein (UDP) are the two components that define dietary crude protein. The relationship between the quantity of microbial protein produced and ME present is uniform. If energy available is less than RDP, then the extra N is converted into ammonia and not recaptured by the microbes. This will cause rumen to absorb it through its wall and convert it urea in the liver. A considerably amount of this blood urea is flushed through urine and as a result is wasted, however some is recycled back into the rumen as salivary urea. (Moran, 2012). A portion of feed protein that passes directly to the abomasum for acid digestion is required in order to ensure that feed protein is efficiently used by calves (Soriano, 1998). Research has shown that the degradability of dietary protein can influence the composition of live weight gain (Moran, 2012). For calves consuming feed with enough energy and total CP, but with low RUP intakes, their growth rate may not be reduced but calves will have more of the live weight gain which consists more of fatty tissue than muscle tissue. Excess fat tissue in the developing udder can reduce its potential to produce milk and later life (Nor et *al*., (2013).

2.2.4. Fibre

The secretion of saliva during chewing and rumination is fuelled by feeds rich in fibre. The saliva contains sodium bicarbonate, which act as buffer to prevent pH from decreasing below critical levels (Joubert, 2012). Sodium bicarbonate also helps with maintenance of normal rumen microbial growth and development (Govil et *al*., 2017). Rumen development can be reduced by feeds that are finely grind as it alters only the physical nature of the fibre, not the chemical analyses. This results in reduced ruminal pH (Daneshvar et *al*., 2015) and can cause excess keratinization of cells as well as ruminal papillae clumping (Greenwood et *al*., 1997). Provision of forage to young calves early in life has been shown to improve solid feed consumption

(Castells et *al*., 2012), which is well utilised when mixed with concentrates to ensure consumption of both feedstuffs without separation (Nemati et *al.*, 2016).

2.2.5. Minerals and vitamins

Calcium (Ca) and phosphorus (P) are two main crucial minerals in the animal body. These minerals play a vital part in the development and maintenance of animal's bone (Albu and Radu-Rusu, 2012). The quantitative relation between these two minerals in feeds, within certain limits concentration, can determine whether the animal will develop bones which are normal or not (Bethke et *al*., 1932). Albu and Radu-Rusu, (2012) and Harty, (2014) reported that the ratio of Ca/P in feed ration of dairy cows is 2:1 or 1:1. Other functions of these minerals are in muscle function Ca and energy metabolism P (Soriano, 1998). Some mineral elements are derived from soil and soil plays an important role in mineral contents in plants (Fleming, 1973). Mineral concentration in feed is affected by plant species, environmental and management factors (Schoombie, 2015). Minerals such as Ca, P, Na, C1, K, Mg, and S are identified important macro minerals (NRC, 1989). Microminerals such as Fe, Mn, Cu, Zn, Se, Co and I are also important for proper bone and cartilage formation which has a direct effect bone growth in young animals.

Vitamin balance is important to attain optimal reproduction, production, health, immunity, and growth (Uys et *al.*, 2011). Vitamins serve as antioxidants and are vital to the metabolic process in ruminants. Neonates receives their vitamins and minerals in colostrum. Supplementation of vitamins (especially B-vitamins and C-vitamins) during stress periods is recommended (Queigley and Drewry, 1998). During the stress periods, addition of vitamins such as B-vitamins and C-vitamins are recommended (Queigley and Drewry, 1998).

2.3. Effects of liquid feed volume on calf growth

In most cases, a dairy farmer will feed calves limited amount of milk because of cost and with a perception that an increase in milk consumption will cause a high occurrence of diarrhoea, reduction in calf starter intake and reduction in body weight gain (Jasper, 2002). Contrary to this perception, research has shown that feeding calves more milk or high-quality milk replacer is not necessary the cause of

diarrhoea (Jasper, 2002; Vieira et *al*., 2008). The cause of diarrhoea is mainly due to the high presence of pathogen in the area, the amount of stress, and the calf's immune system development (Albright, 1997). A study by Jasper, (2002) and Kuehn et *al*., (1994) reported that during the milk-feeding period, high milk intake will decrease consumption of solid feed intake. This will delay weaning as calves will be consuming less dry feed and increasing total amount of milk replacer fed and increasing production cost (Hammell, 1988).

Keeping a calf with its dam will enable calf to suckle seven to ten times a day on average (Bar-Peled, 1997), and consumes additional milk and increase weight gain than original calves raising method (Uys, 2008). Research reported that calves that were fed high amount of milk through suckling showed benefits of early conception and higher amount of milk production (Hammell, 1988). This method enables calves to use its natural sucking behavior (De Passille, 1997) and may increase digestion and improve weight (Trulla, 2007). Unrestricted consumption of milk through a bottle teat is another method of increasing milk consumption.

2.3.1. Enhanced (accelerate growth) nutrition programmes

To achieve growth rate of 500 g/day, calves should be fed 10% of their body weight twice a day (Jaster, 1992). However, attaining greater growth rates might be profitable specifically in early stages of life (2-3 months). Appleby et *al*., (2001) reported that more average daily gain (ADG) can be obtained when feeding milk or milk replacer at accelerated rate. Enhanced or accelerated growth feeding program reduces time needed for calves to reach target body weight as the ADG is greater during pre-weaning period (Appleby et *al*., 2001).

Drackley (2008b) reported that the value of enhanced early nutrition, showed that if nutritional status is improved in the first 2-3 weeks, calves could reach breeding age sooner, improve its ability to resist diseases and increase milk successive production. Accelerated feeding system allows calves high liquid feed intake during early life, this means calves will have unrestricted access to milk. Milk feeding is double compared to the restricted feeding system (Drackley, 2008b). The restricted-feeding system put limits on the amount of milk or milk replacer fed to calves, while

feeding calves unrestricted feed starter on the first week of life. This means the proportion of liquid feed to unrestricted starter intake will be less (Heinrichs, 2014).

2.3.2. Rumen development in accelerated growth calves

Calves are born without rumen, their life starts as a simple-stomached animal and they undergo a process of changes in their digestive tract such as developing rumen which mostly depend on fermentation (Amaral-Phillips, 2006). The reticulum, rumen, omasum and abomasum are the four-part compartments in the cow's stomach system. A big fermentation vat is made up of first two compartments, water and minerals from digesta leaving the rumen are absorbed by the third compartment and the fourth compartment is the true stomach, which functions like stomach of monogastric (Queigley, 1997). The reticulum, and rumen are undeveloped in newborn calves (Queigley, 2001). Rumen develops during first 4-8 weeks after parturition and when calf come in contact with bacteria through animals that it comes in contact with and also bacteria found on feeds (Grobler, 2008). Table 2.3 below indicates development of rumen stomach at different stages.

Table 2.3. Composition of the ruminant stomach at various ages (Soberon, 2012).

Compartment % of total	Birth	28 days	56 days	84 days
Rumen	31	63	71	72
Reticulorumen	35	52	60	64
Omasum	13	12	13	14
Abomasum	49	36	27	22

Adapted from Church (1976)

A slow change in the rumen occurs during weaning when liquid diet is changed to solid diet. After weaning, there is an increase in dry matter consumption and rumen pH, the volume of production volatile fatty acids increases (Soberon, 2012). The VFA production lowers the pH of the rumen and creates a good environment where continuous growth of bacterial is prevented, particularly starch digesting bacteria and propionic and butyric acids production. Carbohydrates in the calf starter feeds (grain mixtures) or forage are fermented to produce propionic and butyric acids. The acetic acid is the end-product when forage or grain mixtures are digested by different

species of bacteria that digest fibre (Queigley, 2001). Feed such as dry feed remains in the rumen for longer and encourages rumen development process.

2.3.2.1. The effects of unrestricted milk feeding on the growth and health of Jersey calves.

The key emphasis of unrestricted milk feeding is on nutrient requirements for the development of young calves. Calves just like other animals they need nutrients for maintenance and development. In addtion, nutrient requirement is not constant, but differs according to body weight, ADG of BW (Heinrichs, 2014) and animal physiological stage. For faster growth, calves need more milk or milk replacer intake while older calves require more starter feed. Contrarily to conventional feed, the accelerated feeding system allows calves to consume more liquid feed in early stages of life, near to normal situations where calves would have *ad libitum* access to milk (Drackley, 2008b). The rates of milk feeding roughly doubles those of the conventional systems (Heinrichs, 2014). A simple feeding method is to supply milk solids in week one of life precisely 1.5% of BW, then increased to 2% of BW in week two until a week prior to weaning (Stamey et *al.,* 2005). Milk replacers that are used in transitional programs generally contains 25 % CP and are fed at approximately 1.5 BW (Heinrichs, 2014).

Jasper (2002) indicated that calves fed on conventional systems delay intake of starter, however, it increases at almost similar speed after the quantity of liquid is cut-off. The feeding programs that have been established are of transitional in nature and they are faster than the conservative programs (Stamey, 2006). However, their implementation is not easy because they do not take full benefit in the growth prospects. Improving nutritional status during early age can allow the calf to resist against infectious challenges (or diseases), and lead to improved growth and increased milk production late after calving (Hoseyni et *al*., 2016; Zahmatkesh et *al*., 2018). Queigley (1997) reported that an improved calf's weight gain during pre-weaning was an effect of Ad lib milk feeding which decreases solid feed consumption.

2.4. Long-time effects of early life nutrition

There is a correlation between dairy cow nutrition and management during early age of life which is potentially altered by metabolic programming influence (Soberon et *al*., 2012). Colostrum management and energy intake pre-weaning have been identified as the two main management practices that have the greatest impacting on future performance (Soberon, 2012). Future production of the dairy herd can be impacted negatively by compromised growth and health of calves (Uys, 2008). Prolonged illness early after birth increases veterinary cost and results in low milk production during lactation.

2.4.1. The development of mammary gland and milk production

The effects of improved levels of pre-pubertal nutrition on successive milk production depends highly on the time of improved nutrition and on the composition of growth (Woof & Burton, 2004). Studies indicated that additional pre-pubertal energy consumption can negatively affect the development of the mammary parenchyma (Grobler, 2008; Soberon, 2012). Mammary gland growth was first described as isometric, followed by an allometric, followed by another isometric stage of growth from puberty to conception, and finally, an allometric growth phase 12 during pregnancy (Sejrsen, 1998). However, the weakness of this study was that nutrient consumption before weaning was not considered.

Studies reports the importance of mammary cells in the lactating gland for production of milk (Husveth, 2011). After parturition, mammary cells continue to increase in numbers, weights and total DNA contents (Uys, 2008). In some species, the influence of this weight increase on milk production can be substantial.

2.5. Direct-fed microbial (DFM) supplementation in dairy calves

Direct-fed microbials (DFM), or probiotics, are living cultures of microbial feed chosen to advance positively the intestinal microbial balance in the host animal (Birkelo, 2003). In the cattle industry, the two commonly used form of DFM are lactate-utilizing and lactate-producing bacteria (Beauchemin et *al.,* 2006). *Propionibacterium*, is a commonly used form of lactate-utilizing bacteria and

Lactobacillus and *Enterococcus* are all forms of lactate-producing bacteria (Elam et *al.,* 2003). The importance of DFM use is increasing fast because of antibiotic usage concerns in animal production. The use of DFM allows the re-establishment of normal gastrointestinal microflora and a decrease in illness related to stress and improve the efficiency of growth in healthy cattle (Beauchemin et *al.,* 2006). In ruminants, probiotics might improve microbial conditions in the gastro-intestinal tract (Kocyigit, 2015). Additional advantages include improved performance, a decrease in diarrhoea prevalent in calves and reduction in shedding *E. coli*.

Seo et *al*. (2010) and Krehbiel et *al*. (2002) reported that feeding DFM to dairy cows can change intestinal microbial populations, enhance disease resistance as well as improve health and performance such as dry matter intake, milk yield, fat-corrected milk yield, and milk fat content. On the contrary, feeding Lucerne (*Medicago sativa)* silage with *Lactobacillus acidophilus* did not affect DMI, milk production and composition in dairy cows, however, a 7.1% improved milk production was reported (Colenbrander et *al*., 1988). This suggest that *L. acidophilus* might not have lived in the silage. The application for DFM in feedlot cattle has improved ADG as well as improve forage productivity (Ware, 1988). Though there was no change observed in DMI, steers treated with DFM gained 7.5% in ADG early in feeding (Kocyigit, 2015).

In a study by Galyean et *al*. (2000), a better final BW on steers treated with DMF was observed than on control animals. The main objective in dairy calf nutrition is to improve the formation of ruminal and intestinal microorganisms by adjusting fast to solid feed and avoid the establishment of enteropathogens, which usually results in diarrhoea (Nock and Kautz, 2006). Newborn and stressed calves are extremely sensitive to rapid diet change or environment which causes changes in microbial population in the gastro intestinal tract (GIT) (Nock and Kautz, 2006).

Feeding bacterial DFM to livestock was motivated earlier by its potential positive post-ruminal effects (Fuller, 1999). However, some bacterial DFM were observed to have positive impacts such as prevention of ruminal acidosis (Ghorbani et *al*., 2002). The characteristics of the latter condition are ruminal pH with less than 5 and high total VFA or lactic acid concentrations. In the presence of Lactate-producing bacteria (*Lactobacillus and Enterococcus*), ruminal microorganisms adapt to the presence of

lactic acid in rumen, which helps to inhibit ruminal acidosis in dairy cows (Kung and Hession, 1995; Yoon and Sten, 1995). (Tannock, 1983) reported that highly fermentable substrate can possibly be utilized to prevent lactate accumulation throughout Inoculation of in vitro fermentation with lactate-utilizing bacterium *Me*.

Microbial cultures were used as an alternative to antibiotics with the sole purpose of improving growth in neonatal and stressed calves and also to increase milk production in lactating dairy cows (Krehbiel et *al*., 2002). While there are many positive responses on DFM, lack well defined plain mechanisms and not clearly understood are a setback. The efficiency of bacterial DFM in neonatal dairy calf have been investigated broadly. Bacterial DFM such as *Lactobacillus* species plays an important role in young calves by establishing and maintaining normal intestinal microorganism (Krehbiel et *al*., 2002). The microbial population in neonates and stressed calves is evolving and very sensitive to change of the diet or the environment (Muya, 2016). Previous report showed a stress can cause high rate of diarrhoea in neonates, in connection with a decrease in Lactobacillus population in the gut (Tannock, 1983). For healthy animals, the faecal counts of *Lactobacilli* are usually higher than coliforms and vice versa for animals suffering from diarrhoea (Sandine, 1979).

In dairy calves, bacterial DFM that have been investigated are mostly Lactobacillus, Enterococcus, Streptococcus and Bifidobacterium species (Seo, 2010). *M. elsdenii* and *Prevotella bryantii* are the bacterial types that have also been used as DFM in order to improve rumen function (Khan et *al.,* 2016).

2.5.1. Mode of action of direct fed microbials

Seo et *al.* (2010) described the role played by direct-fed microbial (DFM) in the rumen. Bacterial DFMs have positive effect in assisting the post-ruminal gastrointestinal tract. These DFMs are possibly more valued possessions of post-ruminal gastrointestinal tract. In Table 2.4 below, a brief summary of modes of action of DFM in the rumen is outlined.

Table 2 4. Mode of action of DFM in the rumen (Seo et *al*., 2010)

Lactic acid producing bacteria
1. Provide constant lactate supply
2. Adaptation of microflora to the lactate accumulation
3. Stimulate lactate-utilizing bacteria
4. Stabilization of ruminal pH
Lactic acid utilizing bacteria
1. Conversion of lactate to other VFA (e.g., *Megasphaera elsdenii*)
2. Production of propionate rather than lactate (e.g., *Propionibacterium* spp.)
3. Increase efficiency of feed conversion
4. Decrease methane production
5. Increase ruminal pH
Fungal DFM
1. Reduction of rumen oxygen
2. Prevention of excess of rumen lactate
3. Provide growth factors (e.g. organic acid and vitamin B)
4. Increase of rumen microbial activity and numbers
5. Improvement of end products of ruminal fermentation
6. Increase digestibility of feed nutrient in the rumen

The reported actions of useful DFM include: Attachment to the intestinal mucosa, inhibition of the growth of possible pathogens, sustaining lesser pH level in the GIT, production of antibacterial compounds (bacteriocin and hydrogen peroxide), alter immune cells and fuel immune role and change the microbial balance in the GIT (Seo et *al*., 2010).

Below is a list of mode of action of DFM post-rumen gastro-intestinal tract as stated by Seo et *al.* (2010):

1. Generation of antibacterial compounds (acids, bacteriocins, etc…)
2. Competition with pathogens for colonization of mucosa colonisation and nutrients
3. Production and stimulation of enzymes
4. Promoting immune response

2.6. *Megaspharera elsdenii* in dairy calves

Megasphaera elsdenii is defined as anaerobic gram-negative, non-motile coccus that ferments soluble sugars and lactate (Piknova et *al*., 2006). Its first isolation was from the sheep's rumen and it is mostly found in young animals' rumen and in those animals receiving feed rich in starch (Stewart and Bryant, 1988). In addition, its presence in humans' and pigs' intestinal contents is well documented (Giesecke et *al*., 1970; Marounek et *al*., 1989). *Megasphaera elsdenii* is the most prominent lactate-utilizing bacterium, utilising up 97% of ruminal lactate (Counotte et *al*., 1981; Miller, 2013; Muya, 2016). In addition to its role in lactate fermenting, *M. elsdenii* plays a crucial part in the making of branched-chain VFA in the rumen (Thieszen et *al*., 2015; Muya, 2016). Therefore, it has been evaluated as a DFM for prevention of acidosis (Miller, 2013).

In a study of an *in vitro* and *in vivo* by Kung and Hession (1995), the outcome showed that *M. elsdenii* was capable of modifying ruminal fermentation and stop lactate build-up throughout a low to high diet transition. A study by McDaniel (2009) reported that cannulated steers had greater ruminal pH and lesser lactate concentrations when fed a high concentrate diet and inoculated with *M. elsdenii* compared to those that did not receive *M. elsdenii.* Dosing *M. elsdenii* lactating dairy cows showed a decrease in time period of ruminal pH< 5.6 compared to cows that did not receive it (Miller et *al*., 2013). When *M. elsdenii* is administered to cattle prior to introduction of high-concentrate diet, it showed an increase in DMI, ADG and improved efficiency in feedlot cattle (Aikman et *al*., 2009). If similar administering (*M. elsdenii)* was done on dairy cattle, an increased in DMI, milk production and milk production efficiency was observed (Aikman et *al*., 2011; Henning et *al*., 2011).

When cultures were treated with *M.* elsdenii, the pH decreased beneath 5.5 in 4 hours and remained approximately at 5.3 (for 24 hours culture) although the control's pH drop to 4.8 (Kung, 2015). After 8 hours, in the same experiment, lactate concentration reached a peak of more than 40 mM in control and stayed fairly the same afterwards, however in *M. elsdenii* treatment less than 5mM of lactate concentration was observed. The total VFA concentration of *M*. *elsdenii* treated cultures was recorded to be more than twice (131.4 vs. 63.3 mM) that of control.

After 2 hours, no significant difference was observed in the acetate concentration. At 6 hours, the observed concentration of propionate butyrate, valerate, isobutyrate and isovalerate did not vary in both the control and *M. elsdenii*. Kung (1995), this led to a conclusion that the greatest variance in VFA concentration between treatments was due to an increase in butyrate, valerate, and branched-chain fatty acids,

CHAPTER 3
MATERIALS AND METHODS

3.1. Data collection

A set of data on the performance of heifers during the pre- and post-weaning period, as well as their milk performance, was obtained from the dairy herd of the Agricultural Research Council–Animal Production. Heifers were weighed at birth, and when 42 days, 70 days, 15 months old. Data included 11 calves that received and 11 that did not receive *Me* early after birth from March 2012 to January 2014. The *Me* treated calves were dosed 2 weeks after birth. Data on milk performance (yield and composition) for the entire first lactation for the same heifers was obtained from the Alpro Herd Management System (DeLaval. (Pty) Ltd, Heilbron, 9650, South Africa), a system that record and store milking information. The same data was confirmed with data on the National Dairy Records Scheme through INTERGIS system. Heifer's data on BW and age at first insemination, number of inseminations were obtained from the ARC dairy herd. Heifers were eligible for insemination at 15 months of age and had attained body weight of approximately 375 kg. Calves were allocated into two groups (dosed and not dosed with *Me*) in a complete randomised block design according to birth date.

3.1.1. Calf feed intake

Daily intake of calf starter was determined from amount fed minus orts and recorded.

3.1.2. Animal body weight

The calves were weighed at start and thereafter every week until 2 weeks after weaning to determine the average daily gain (ADG). After the first 42 days, heifers were weighed every 2 weeks until 70 days of age before being weighed every month. Growth parameters (ADG and BW) were determined from weekly body weight measurement.

3.1.3. Number of inseminations

Heifers were first inseminated at 15 Months of age. The pregnancy diagnosis was performed after 2-3 months and repeated if not successful, and the age at first insemination (in month) as well as the number of inseminations were determined in association with the animal BW and recorded.

3.1.4. Milk yield and composition

At the start of lactation, the daily milk yield was recorded, and milk sample collected every week for the determination of milk fat, protein and lactose.

3.2. Data analysis

For the effects on pre- and post-weaning calves performance as well as milk yield, data were analysed as repeated measures using the PROC MIXED model of SAS (Statistical Analysis System, 2012). Feed intake and milk yield were measure daily and data were pooled by week (for feed) and month (for milk) for the analysis. The statistical model included calf and cow as a random effect, and experimental group and its interaction with time as a fixed effect. Results are presented as least square means.

The statistical model used for repeated measure analyses was

$$Y_{cit} = \mu + \alpha_i + \beta_t + T_{it} + \delta_{ci} + e_{cit},$$

where Y_{cit} = an observation value measured from animal c from treatment i at time t;
μ = overall mean for the population;
α_i = fixed effect of treatment i, where i = Me0 or Me14;
β_t = fixed effect of time t;
T_{it} = fixed interaction of effect of treatment i and time t;
δ_{ci} = random effect of animal c nested within i treatment; and
e_{cit} = error associated with each Y_{cit}

For the effects on Birth BW, Weaning BW, BW on day 70, month 15 and age at first insemination, data were subjected to ANOVA using PROC GLM (SAS Institute, 2012). Results are presented as least square means. The statistical model below was used:

$Y_{ci} = \mu + T_i + \delta_c + e_{ci}$

Where Y_{ci} = observation value taken from animal *c* at *t* time.
μ = overall mean of the population,
T_i = fixed effect of the i^{th} treatment (Control or Me),
δc = random effect of animal, and
e_{ci} = error associated with the measurement taken from animal *c* from i^{th} treatment

Significance was declared at $p < 0.05$.

Mixed-effects linear regressions were performed to establish relationships between calf's ADG and weaning BW as well as milk yield and new calf birth weight were assessed using SAS (2012). The correlation coefficient (Pearson) also determined to assess the association between the above-mentioned parameters.

CHAPTER 4
RESULTS

4.1. The effects of dosing *M. elsdenii* on heifer's milk and starter feed intake during pre- and post-weaning period

Least square means for the daily average of milk intake, starter, and total dry matter intake, and metabolisable energy intake for both the pre- and post-weaning period are presented in Table 4.1. Milk, pre-weaning starter and metabolised energy intake did not differ between the control and the *Me* groups. Post-weaning starter feed intake was higher ($p=0.03$) for *Me* fed heifers than control heifers. The post-weaning metabolisable energy intake was also higher ($p=0.03$) for heifer fed *Me* than control heifers. There were significant effects ($p<0.001$) of time on all intake parameters, but only the pre-weaning starter DMI showed interaction ($p<0.05$) between treatment and time. Pre-weaning transformation indexes was greater for Me-heifers than control heifers.

Table 4.1. Milk, dry matter and energy intake of calves dosed (Mega) or not (control) with *M. elsdenii* (Least square mean)

Parameters	Treatments		SEM[1]	P-value		
	Con	Mega[2]		T[3]	Time	T x Time
Intake						
Milk intake, Lit/d	8.25	7.98	0.44	0.67	<0.001	0.44
Pre-weaning starter DMI[4], kg/d	0.11	0.13	0.02	0.04	<0.001	0.01
Total pre-weaning DMI, kg	1.13	1.11	0.05	0.85	<0.001	0.71
Post-weaning starter DMI, kg	1.52	1.91	0.11	0.03	<0.001	0.59
Water intake, Lit/d	4.26	4.15	0.27	0.66	<0.001	0.62
Ratio water/Total DMI, Lit/kg	3.77	3.74	0.44	0.63	<0.001	0.57
Transformation index[5]	1.88	1.52	0.25	0.03	-	-
Metabolised energy intake, Mcal/d						
Pre-weaning	5.40	5.30	0.25	0.77	<0.001	0.66
Post-weaning	4.82	6.08	0.34	0.03	<0.001	0.59

[1]Standard error of mean

[2]Megasphaera elsdenii

[3]Treatment

[4]Dry matter intake

[5]calculated as the ratio average total dry matter intake: average daily gain

4.2. Influence of dosing *M. elsdenii* on heifer's average weight gain during pre- and post-weaning

The average daily weight gain was measured during the pre-weaning period and after weaning and results are showed in Figure 4.1. The average daily weight gain of heifers dosed with *Me* was higher during the pre-weaning period (0.66 kg/day; p=0.04) and after weaning (1.12 kg/day; p=0.03) compared to control (0.60 and 0.65 kg/day, respectively).

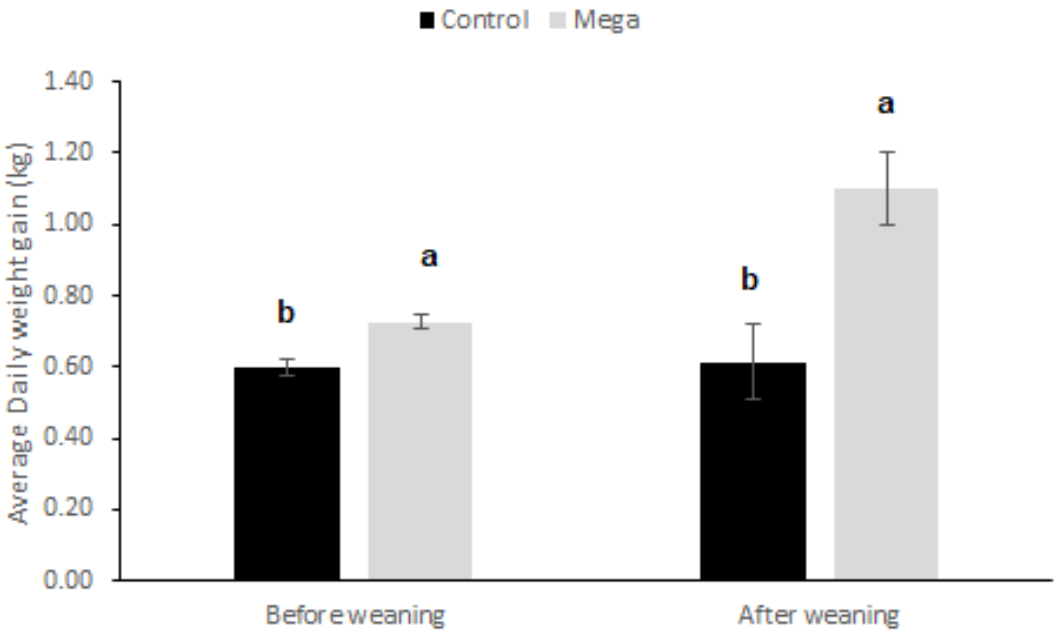

Figure 4.1. Average daily gain during pre- and post-weaning for control and Mega heifers. Mega: Heifers that were dosed with *M. elsdenii* during the pre-weaning period. [ab] Means treatments with different superscripts within measurement period (birth, weaning or 2 weeks after weaning) differ ($p<0.05$).

4.3. Effect of dosing *M. elsdenii* on heifer's body weight change

The heifers increased BW with age and approximately doubled the initial BW at 70 days old and had 11 times more weight at 15 months old. At 42 and 70 days old, the BW of *Me*-heifers was greater (75.8 ± 2.6 and 91.2 ± 4.6 kg) than control heifers (61.9 ± 2.6 and 77.2 ± 4.6 kg) ($p<0.05$). When the heifers were 15 months old the average BW in both groups was 379 ± 10.2 kg and did not differ ($p>0.05$) between the groups.

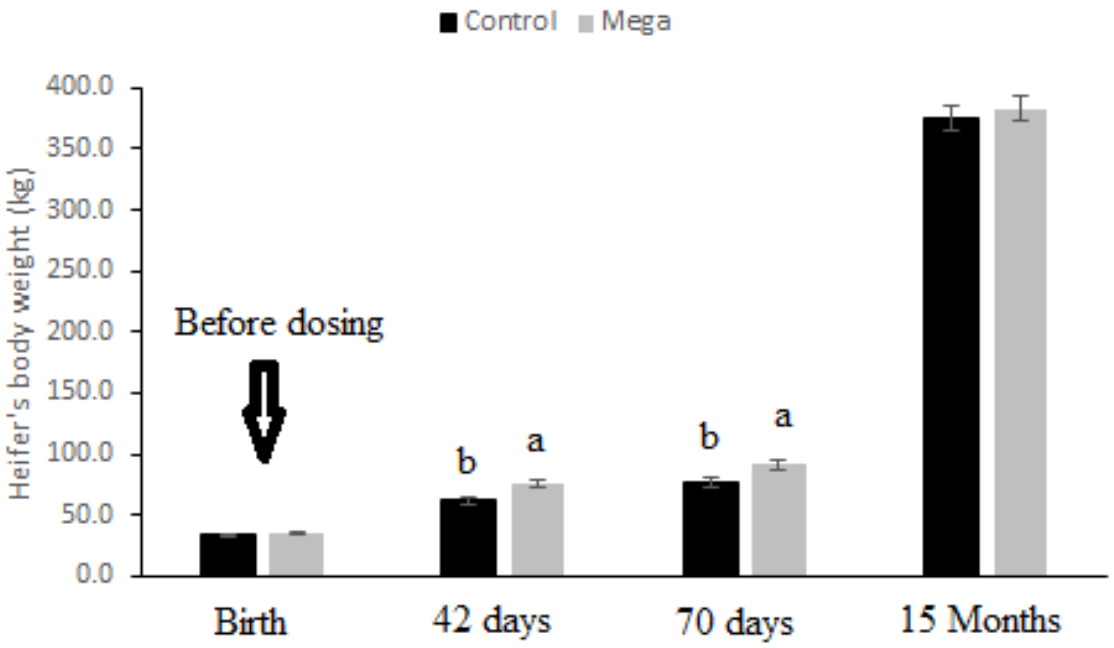

Figure 4.2. Change in body weight for control and Mega heifers. Mega: Heifers that were dosed two weeks after birth. [ab] Means treatments with different superscripts within measurement period (birth, weaning or 2 weeks after weaning) differ ($p<0.05$).

4.4. Age and number of inseminations as affected by dosing *M. elsdenii*

The results on the effects of *M. elsdenii* on age, BW and number of inseminations are presented in (Table 4.2). There was no difference ($p>0.05$) in BW at insemination and number of inseminations between the control and supplemented heifers. However, the average BW at insemination was found to be 379.5 kg and the average number of inseminations was 1.25.

Table 4.2. Least square means for the average age, BW, and number of inseminations as affected by dosing *M. elsdenii*

Parameters	Treatments		SEM[1]	P-value
	Cont	Mega		
Age	15.1	15.2	0.02	0.49
BW at insemination	375.7	382.6	14.4	0.51
Number of inseminations	1.2	1.3	0.09	0.63

[1]Standard error of mean

[2]*Megasphaera elsdenii*

4.5. Influence of dosing *M. elsdenii* on heifer milk lactation and composition

The least square means for the average milk yield (kg/day), milk nutrient (fat and protein) concentration (%) nutrient fat yield (kg/day) and milk nutrient (protein and energy) measured during the 10 months of the first lactation are presented in Table 4.3. There were no differences in milk yield, milk fat %, milk protein, fat yield, and milk energy between the two groups of cows ($p>0.05$). These milk parameters averaged 25.2 kg/day, 3.45%, 0.86 kg/day, 0.79 kg/day and 25.1 kg/day for milk yield, milk fat %, milk protein, fat yield, and milk energy respectively. The percentage of protein in milk was 2.8% higher ($p=0.04$) in *Me* (3.22%) than in control cows (3.13%). There were effects ($p<0.05$) of time on milk yield and milk fat (% and kg/day), and interaction ($p<0.05$) between treatment and time for milk protein (% and kg/day).

Table 4.3. Effects of dosing *M. elsdenii* on milk yield and composition (least square means)

Parameters	Treatments		SEM[1]	p-value		
	Control	Mega[2]		T[3]	Time	T x Time
Milk yield, kg/day	24.8	25.6	1.46	0.89	0.03	0.23
Milk fat %	3.46	3.45	0.02	0.76	0.02	0.19
Milk protein %	3.13	3.22	0.05	0.04	0.09	0.03
Milk fat yield, kg/day	0.86	0.87	0.05	0.91	0.03	0.21
Milk protein	0.78	0.81	0.04	0.69	0.07	0.03
Milk energy, E	24.8	25.4	1.45	0.85	0.06	0.09

[1]Standard error of mean

[2]*Megasphaera elsdenii*

[3]Treatment

The total milk yield is presented in Figure 4.3. The total first lactation milk yield is 7632.3 ± 359.3 kg and no difference was observed between the control and *Me*-heifer groups ($p = 0.54$). The change in milk over time during the first lactation period is shown in Figure 4.4. The change in milk yield over time was similar in both groups of heifers, increasing from calving to month 3, remaining static from month 3 to 4 and slightly decreases from month 4 to 7 and rapidly decreases from month 7 till dry off (month 10). No difference in milk yield was observed during the whole first lactation period

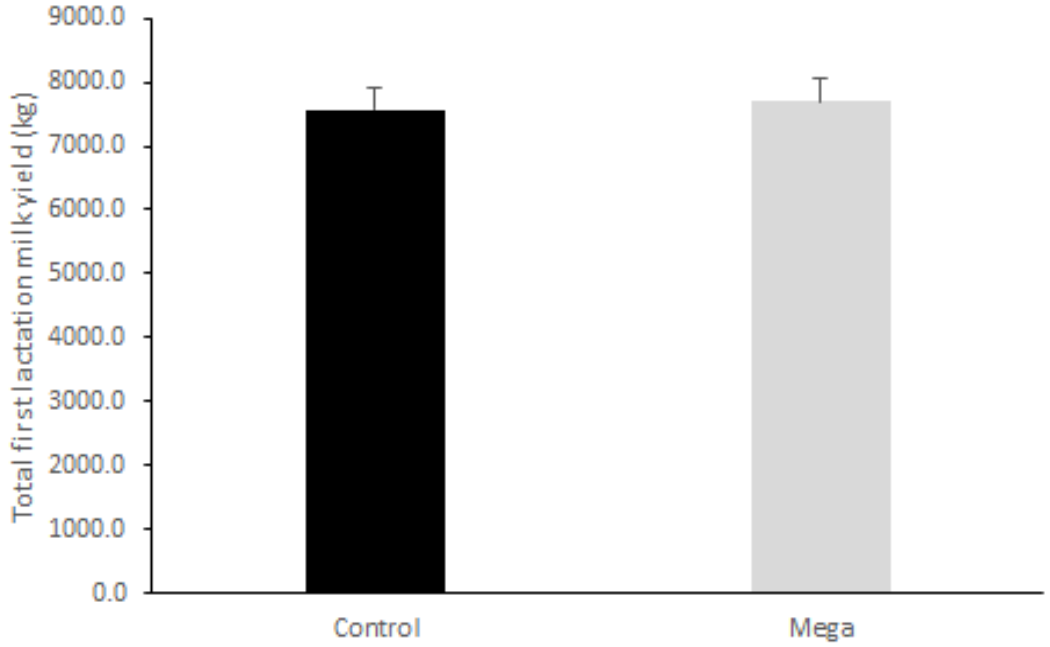

Figure 4.3. Total first lactation milk yield (± SE) for Mega and control cows. No significant difference (p = 0.54). Mega: Cows in the group the received *M. elsdenii* during the pre-weaning period

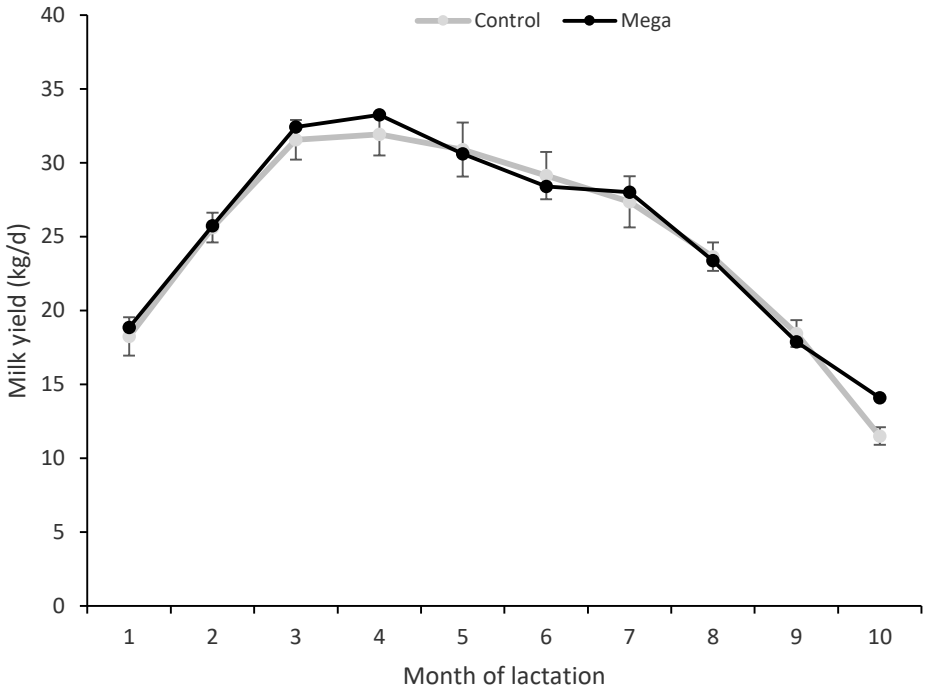

Figure 4.4: Change overtime of the total first lactation milk yield (± SE) for Mega and control cows. Mega: Cows in the group received *M. elsdenii* during pre-weaning period.

The milk protein percentage during the whole first lactation for Mega and control groups is presented in Figure 4.5. During the whole first lactation in both groups and did not differ between the two groups, but control milk protein remained above in the *Me* fed cows. Only in month 4 and 5, when milk protein percentage were lower in *Me* fed cows than control cows.

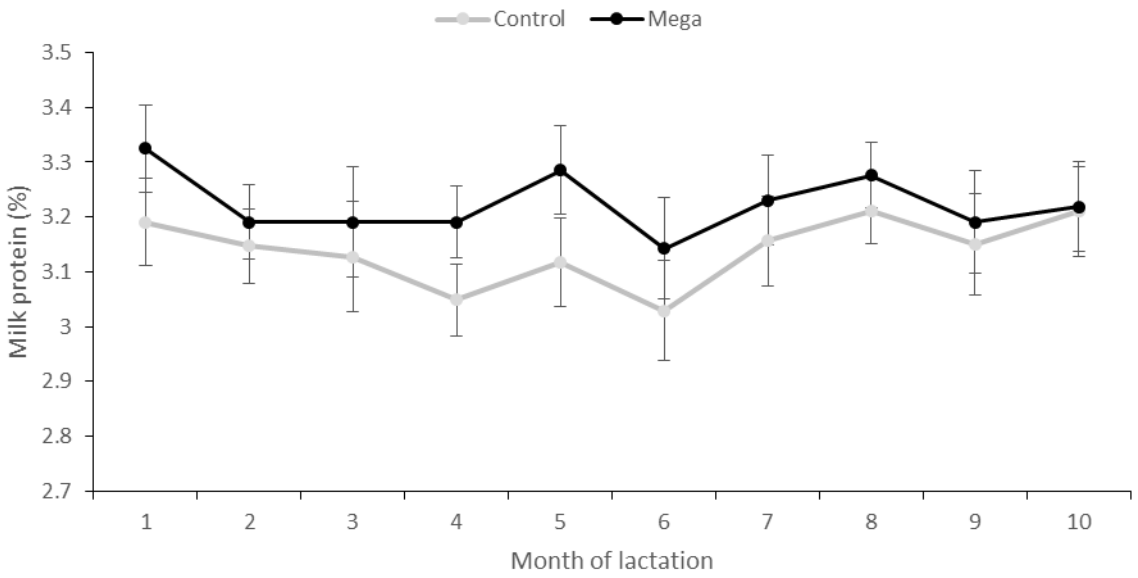

Figure 4.5. Change overtime of the milk protein percentage (± SE) for Mega and control cows. Mega: Cows in the group the received *M. elsdenii* during the pre-weaning period.

As for milk fat percentage, there was a fluctuation of milk fat percentage throughout the whole first lactation period in both groups. The linear trend line (Figure 4.7) for the milk fat during 10 months of the first lactation indicated a decline in milk fat percentage from 3.51 to 3.46% for control group and from 3.52 to 3.36% for the *Me* group.

Starter DMI was very low in the control and Me-treatments but increased exponentially after day 52. This was associated with milk withdrawal, as it was decreased to 4L fed once in the morning until weaning. The differences between treatments in starter DMI became obvious from week 7.

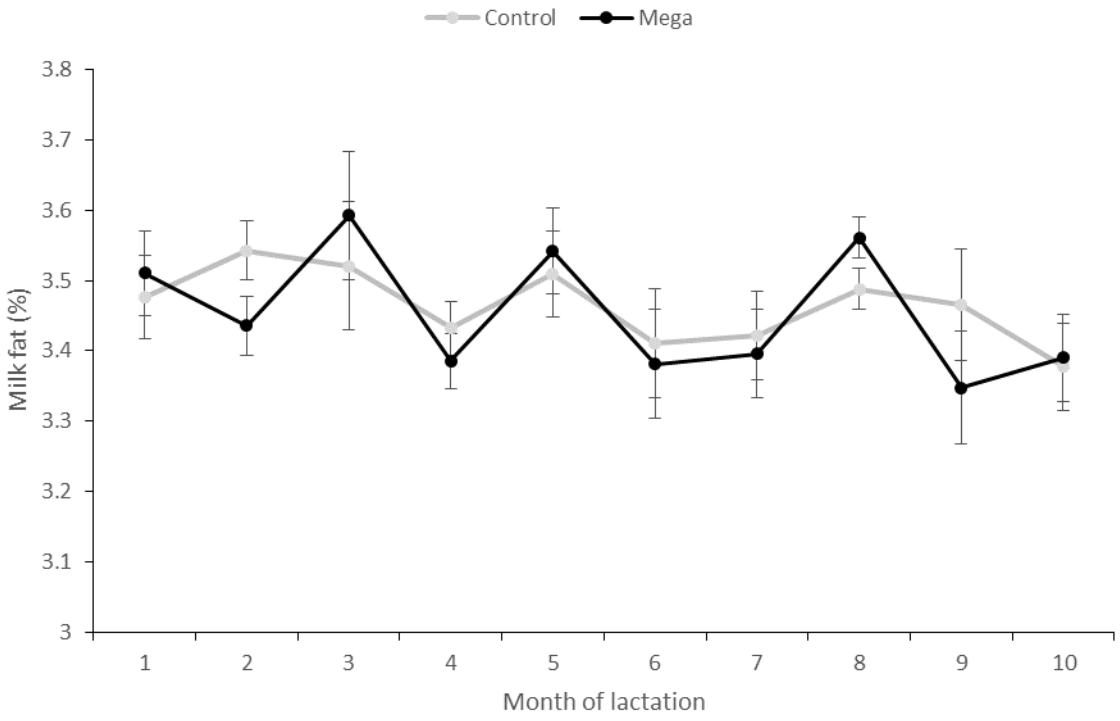

Figure 4.6. Change over time of the milk fat percentage (± SE) for Mega and control cows. Mega: Cows in the group the received *M. elsdenii* during the pre-weaning period.

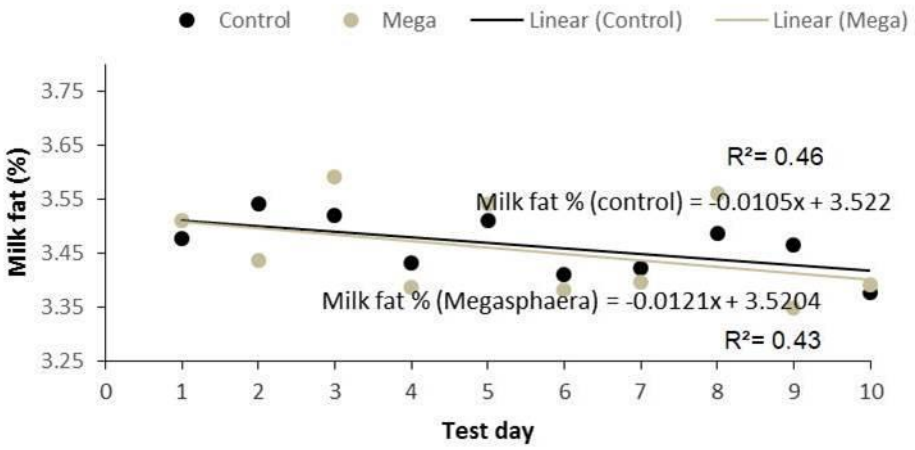

Figure 4.7. The trend of the milk fat percentage (± SE) for Mega and control cows. Mega: Cows in the group the received *M. elsdenii* during the pre-weaning period

4.6. Relationship between growth parameters and heifers' BW at conception and total milk

The relationship between measured parameters were established and presented in Table 4.4.

Table 4.4. Relationship between growth parameters and BW at conception and total milk

Parameters	Linear relationship		Correlation	
	R^2	p-value	r	p-value
Pre-weaning starter intake and ADG before weaning	0.55	0.002	0.76	0.002
ADG before weaning and weaning BW	0.73	0.003	0.08	0.022
ADG before weaning and BW post-weaning	0.76	<0.001	0.48	0.005
ADG before weaning and first lactation total milk yield	0.12	0.88	0.67	0.09
ADG 2 weeks after weaning and first lactation total milk yield	0.76	0.61	0.76	0.55
ADG before weaning and BW at conception	0.38	4.423	0.08	0.79
ADG 2 weeks after weaning and BW at conception	0.15	11.4	0.14	0.63
BW at conception and first lactation total milk yield	0.98	0.07	0.74	0.09

[1]Average daily weight gain

[2]Body weight

The pre-weaning starter intake (kg/day) and ADG before weaning presented a positive ($p = 0.002$, $R^2 = 0.55$). The ADG before weaning and weaning BW was positively related to the weaning BW ($p = 0.003$, $R^2 = 0.73$) and BW post-weaning ($p < 0.001$, $R^2 = 0.76$). No relationships were observed for other parameters.

CHAPTER 5
DISCUSSION

5.1. Feed intake

Calves in both groups consumed very little starter feed during the milk feeding period because they all had free access to milk. Calves can consume up to 10 L/day of milk if allowed. This was also reported in other studies (Terré et *al*., 2006, Muya et *al*., 2015) and this has been attributed to calves that received more milk can meet their required intake of nutrients from milk. In addition, this resulted in reduced available space and limits amount of solid feed that can be consumed (Muya et *al*., 2015). However, the heifers that received *Me* consumed more starter feed compared to the group of heifers that did not receive *Me*. Miller *et al.* (2013) explained that calves that were dosed with *Me* developed absorptive capacity (Coverdale et *al*., 2004), which may stimulate calves to consume more starter feed, which explain the interaction between treatment and time observed in the present study.

The intake of energy from feed was the same between the two groups of calves, which is an indication that it was not influenced by dosing *Me* during the pre-weaning period in this study. Muya et *al*. (2015) reported that the intake of energy was influenced early after weaning, suggesting that more time may be needed for the effects of *Me* to be expressed. In the present study, only heifers were used, while in Muya et *al*. (2015) study, heifers and bulls were used, which may explain the difference. Water intake did not differ between the two groups, which indicated that despite the tendency of calves fed *Me* to drink less milk, they did not attempt to compensate it with an increased consumption of water as observed in other studies (Terré et *al.*, 2006). Maybe this was because all heifers consumed the same amount of total feed DM.

5.2. Heifer growth

Heifers fed *Me* had higher ADG, probably because they consumed more starter feed, which, according to Terre et *al*. (2006) is caused by greater nutrient digestibility when heifer consume more starter feed (Terre et *al*., 2006). Consequently, calves

fed *Me* had greater BW at time of weaning on day 56, but also on day 70. The improved growth performance with *Me* is confirmed by the great transformation index of *Me*-heifers compared to control heifers. However, no difference was observed at 15 months, when heifers were matured for breeding. To be qualified for breeding, heifers had to reach 375 kg BW at the age 15 months. It was expected that heifers would have better growth due to *Me* effects on feed intake and reached the target BW earlier. All heifers (in both groups) did not show any difference on BW at 15 months and were all inseminated at the same time. This raises a question on how long *Me* survived the GIT after a single dose as applied to these heifers? In a study by Yohe et *al*., (2017), the administration of *Me* had little effects on the rumen microbiome composition, but the rumen microbiome composition showed temporal successions as the calves grew. This study did not observe effects of *Me* after 35 days after dosing though the trial conditions and feed could not be the same, leading to different time of the expression of *Me*. The current results suggest that *Me* may not survive for a long period in the heifer's GIT after a single dose. The dairy heifer calves that achieved greater weaning BW, increased growth rate from birth to weaning, which was associated with earlier puberty ages (Macdonald et *al*., 2007). It was also expected that greater ADG and weaning BW of heifers fed *Me* could result in greater BW at the mature age. Macdonald et *al*. (2007) indicated that the BW influenced more attainment of dairy heifer's puberty than age. This was not observed in this study, which might be associated with the lack of difference on the age at insemination and the number of inseminations between the control and Mega fed animals.

5.3. Heifer lactation performance

The milk production was evaluated during the first lactation period and average milk yield did not differ between cows that received *Me* during the early age (pre-weaning period) and cows that did not receive *Me*. All cows produced an average of 7500 litres of milk, and apart from milk protein that were higher in *Me*-cows, no other measured milk parameter differed between two groups. The milk protein percentage fluctuated during the whole first lactation in both groups and generally did not differ between the two groups, but control milk protein remained above the *Me* cows. Milk composition was, however, affected when *Me* was dosed to cows during their

lactation. Aikman et *al.* (2011) reported a reduction in milk fat and protein when high-producing cows were dosed with *Me* on both their standard and high-energy diets. Different milk fat percentages were reported by Hagg et *al.* (2010) between dosed and control cows fed a high-concentration diet. Milk fat fluctuated between 3.3% and 3.6% but no major difference was observed. It is known that the nutritional status between calf birth and puberty can exert permanent effects on the ability of adult cows to produce milk. Although heifers fed *Me* had greater BW at weaning and 70 days of age, no difference was observed at puberty between these heifers and those not fed *Me*.

Diaz et *al*. (2001) and Bartlett et *al*. (2006), suggested that for every unit of feed nutrient consumption above requirement for maintenance, protein deposit significantly increased. This might be accountable for developmental functions that lead to improved first lactation milk production. It was concluded that as nutrient supply is increased in calves during the pre-weaning period, their potential to improve lactation improved. In the present study, heifers fed *Me* had higher started DMI than control heifers and the total DMI did not differ due to higher milk intake by control calves. This can explain the lack of possible improvement on the first lactation.

When comparing performance of heifer fed either restricted milk replacer, or allowed to suckle, calves that were allowed to suckle gained more weight and yielded more milk during their first lactation due to higher DMI intake before weaning (Drackley, 2005). It was also demonstrated in other studies that increased nutrient supply in neonatal dairy calves potentially increased first and most likely subsequent milk yield (Moallem et *al*., 2010; Lohakare et *al*., 2012). In the study of Soberon et *al*. (2012), the pre-weaning average daily gain (ADG) of calves accounted for 22% of the variation in first lactation milk yield and that more milk was produced by animal that had greater nutrient intake from milk or milk replacer during the pre-weaning period. According to Zanton and Heinrichs, (2005) and Zanton and Heinrichs (2007), a growth rate of approximately 800 g/day from 150 to 320 kg BW is required for maximum first lactation milk and protein yields. Soberon and Van Amburgh, (2014) reported that milk yield in the first lactation was improved by increasing nutrient intake from milk or milk replacer during the calf pre-weaning period. Futhermore,

they reported that for every kilogram of pre-weaning ADG, first lactation milk yield had increased by 1550 kg. It was further concluded that appropriate nutrition in early life can have positive impacts on productive dairy cows. Early rapid growth is beneficial if intra-parenchymal fat content of the mammary gland is not increased.

The observed relationship between the ADG before weaning and weaning BW, and between the ADG before weaning and BW post-weaning justify the observed effects of Me on increased feed intake and growth of heifers before and early after weaning. The present study did not find any relationship between milk performance and early life performance.

CHAPTER 6

CONCLUSION

The target of dairy farmers is to have better growth of replacement heifers to obtain good future milking cows and improve income. Management strategies to produce better dairy animal must also reduce raising cost. *Megasphaera elsdenii* has demonstrated an ability to improve heifers' performance through improved GIT absorption capacity and feed intake. Better early heifer performance has been associated with earlier puberty ages but also improved milk production. Early feeding of *Me* to heifers in the present study positively affected heifer growth during and early after milk feeding period, confirming previous reports. Animal weight at puberty and subsequent milk production were not influenced by feeding *M*. *elsdenii* It is possible that *M*. *elsdenii* did not survive after weaning to continue to express its influence on animal performance.

RECOMMENDATIONS

Based on the results, it is possible that *Me* did not survive long after weaning to continue to express its influence on animal performance. Research is warranted to evaluate the long-term survival of the bacteria.

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