

Effects of *Carica papaya* seed (*Linn*) meal on health and performance of Jersey calves

by

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submitted in accordance with the requirements

for the degree of

MASTER OF SCIENCE

in the subject

AGRICULTURE

at the

UNIVERSITY OF SOUTH AFRICA

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JANUARY 2018

DECLARATION

I, Rudzani Precious Makoya declare that this is my dissertation, "Effects of *Carica papaya* seed (*Linn*) meal on health and performance of Jersey calves", and 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:

Date:

Signature:

DEDICATION

This work is dedicated to my beloved family: my mother (Thinabuya Annah Makoya) and siblings (Ndivhudza Makoya, Mpfareleni Makoya and Nonhlanhla Makoya) for their supports and encouragements throughout the way. In addition to my beloved son, Thendo Mukhakhululi for always motivating me to go further and helped to keep my eyes on the price.

ACKNOWLEDGEMENTS

Firstly, I want to thank **Jesus Christ** for providing me with wisdom and strength. In addition, I acknowledge Him because without Him nothing is possible. For surely I do everything by His grace that is upon me.

I acknowledge and deliberate my sincere indebtedness to the following people and organisations. Who offered encouragement and contributed on the preparation of the dissertation.

My sincere gratitude goes to Professor Khanyisile R. Mbatha from UNISA for her valuable skills, guidance, time, friendship and support during the project.

In addition, thank Doctor Mukengela Claude Muya from ARC for his valuable time, advice and for always listening to my errors and offering to help.

For the generous financial support to the following organisations are gratefully appreciated: University of South Africa and National Research Foundation (NRF-RSA)

My sincere gratitude goes to the Bethel Agricultural College, Eastern Cape for their facilities, and farm staff for their support.

I have pleasure to acknowledge the Agricultural Research Council-Animal Production Institute, Irene (ARC-API), for providing with animals and facilities for the study. Furthermore, to express my sincere gratitude to Ms Kabelo Manyama for assisting with faecal sampling and the support from dairy unit staff for their technical assistance with animals during the data collection.

Finally, I would like to acknowledge my family especially my mother, for their understanding, motivation, constant support, and encouragement along the way. **Ndo livhuwa!!**

ABSTRACT

Twenty four Jersey calves were randomly blocked according to sex and birth date, to determine the effects of supplementing *Carica papaya* seed (*Linn*) meal (CPSM) on health and growth performance of calves. In the first study, calves were fed CPSM for only 2 days to determine faecal pathogen population. In the second study; calves were randomly assigned at birth to three treatments and fed until weaning at 42 days. Each treatment had six calves. Treatments were; 1) a control group, which did not receive additive; 2) a group that was supplemented 5 g/d of *Carica papaya* seed meal (CPSM) and 3) the last group that was supplemented 5 g/d of a commercial product containing *Lactobacillus acidophilus* (Lact). After receiving milk with colostrum from their dams for 3 consecutive days after birth, calves had commercial starter pellet and fresh water *ad lib*. Treatments were added to whole milk from day 4 and fed to calves before being allowed to suckle from the dams for 30 min three times a day (08:00; 12:00 and 17:00). Calf starter dry matter intake (DMI) was evaluated daily while body weight (BW) and body structural growth were measured weekly. Faecal samples were collected directly from the rectum on day 7 and 10, before and after receiving CPSM respectively. The DMI; DMI/BW; BW and BWG of calves did not differ among treatments, and averaged 305.4 g/d; 7.7 g/d per BW; 39.4 kg and 32.1 kg, respectively. The initial BW, 22.2 ± 1.49 kg did not differ among groups, but the weaning BW was higher ($p < 0.05$) for CPSM fed calves compared to control diet fed calves. It did not differ between control and Lact calves. Calves in Lact and CPSM treatments had similar average daily gain (ADG) and heart girth (HG), which were higher ($p < 0.05$) than calves fed the control treatment. Calves fed CPSM had higher ($p < 0.05$) hip width (HW) and shoulder height (SH) than control calves. There were effects of time ($p < 0.001$) for starter DMI/BW, ADG and all structural body parameters, and effects ($p < 0.05$) of interaction between time and treatments for only starter DMI/BW, ADG and HG. The CPSM treatment reduced faecal coliforms and *E. coli* ($p < 0.05$) by 93.6% and 96.1%, respectively; and tended to reduce *Enterobacteriaceae* ($p = 0.056$) by 96.4%. The present study revealed that feeding CPSM to calves during the pre-weaning period increased growth performance by improving average daily gain, feed efficiency and enhancing health status due to low faecal pathogen count.

Keywords: Probiotics, faecal pathogen, growth performance, calves

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CHAPTER 1

INTRODUCTION

The highest rate of neonatal mortality incidence occurs during the first three weeks of dairy heifers' life (Wells *et al.*, 1996; Wudu *et al.*, 2007). Diarrhoea is the key reason of sickness and death in of calves' early life (Svensson *et al.*, 2006; Windeyer *et al.*, 2014; Uetake, 2013). The first peak of respiratory sicknesses is often observed at the age of four weeks, resulting in extensive revenue loss due to growth depression and medication (Postema *et al.*, 1987). This situation is worsened by inadequate acquisition of inert immunity in newborn calves (Donovan *et al.*, 1998; Virtala *et al.*, 1999). This may be caused by poor efficiency of immunoglobins (Ig) absorption, poor colostrum feeding management, and colostrum containing inadequate mass of Ig (Quigley, 2005). This results in the calves prone to diseases, mainly bacterial infections of the digestive tract (McGuirk, 2008).

Usually on dairy farms, newborn calves are fed milk or milk formular containing antibiotics to prevent diseases and improve calf growth (McGuirk, 2008). Nevertheless, the use of antibiotics used as feed supplement is related to the augmentation of antibiotic-resistant strains resulting in their reduced efficacy (Wegener, 2003), which necessitate other alternatives. The benefit of supplementing probiotics on calf performance has been associated with balanced gastro-intestinal tract microbiota and improved calf health (Chaucheyras-Durand & Durand, 2009).

Natural plants and their extracts such as garlick, papaya, pumpkin, contain phytochemicals (secondary metabolites) that exhibit antibacterial activities against a wide range of pathogens and may be used as growth boosters (Baladrin *et al.*, 1985; Githiori *et al.*, 2006). The seeds of *Carica papaya Linn* have therapeutic and pharmacological properties which are anti-amoebia, anthelmintic, antimicrobial and anti-fertility (Osato *et al.*, 1993). Furthermore, seeds are bacteriostatic against enteropathogens such as *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The papaya seeds have medicinal uses such

as carminative, emmenagogue, counter irritant, treatment of ringworms, psoriasis, bleeding piles as well as enlarged liver and spleen (Rupa & Jayanta, 2013).

Problem statement

Calf mortality remains the main problem in farming industry around the world due to calfhood diseases that has a negative impact on growth performance and farm income (Smith & Little, 1922; Mohri *et al.*, 2007). Several signs of sickness and death observed in calves are during routine antibiotic treatment of calf diarrhoea (Lance *et al.*, 1992), in calves born to first lactation heifers and in group housing (Olsson *et al.*, 1993). Furthermore, calves that are born at a difficult calving period and inadequate passive transfer of colostrum are identified to cause morbidity and mortality (Wells *et al.*, 1996). Respiratory diseases remain one of the leading causes of calf sickness and mortality (Woolums *et al.*, 2005), resulting in increased antibiotic usage (Holland *et al.*, 2010) and deficiency of optimal passive transmission of colostrum immunity (McGuirk & Ruegg, 2011).

Calves are fed antibiotics or feed additives in milk and dry feed to reduce prevalence of infection of diseases early in life as well as to improve growth performance (Galvão *et al.*, 2005). The primary effects of antibiotic fed supplements are to enhance opposition to colonization with pathogenic microorganisms and animal health status as well as to enhance mucosa immunity of the animals (Choct, 2009). However, addition of such products has led to increased resistance to antibiotics (Phillips *et al.*, 2004), which necessitate alternative strategies.

Non-antimicrobial feed additives with a possibility to lessen establishment of pathogenic microorganisms of the gastro-intestinal tract such as *Carica papaya* seeds seem to be one of natural alternatives to antibiotic and can be tested as additives in animal feeds. Moreover, these papaya seeds are not commonly used for human consumption.

Objectives

The primary objective was to investigate the performance and health of dairy calves when supplemented with *Carica papaya* seed (Linn).

The specific objectives are to:

- Ascertain the effects of CPSM on selected opportunistic pathogenic bacterial (Coliforms, *Escherichia coli* and *Enterobacteriaceae*) populations in dairy calves.
- Determine the effects of supplementation with CPSM on calf feed intake, growth and structural body parameters of suckling Jersey calves.

Hypothesis

The hypotheses were:

- Supplementation of dairy calves with CPSM would have significant effects on faecal coliforms, *Escherichia coli*, and *Enterobacteriaceae*.
- Supplementation of dairy calves with CPSM would have significant effects on feed intake and calf growth.

To achieve these objectives, two studies were conducted:

Study 1: The effects of CPSM on faecal pathogen populations in dairy calves and its influence on calf performance.

Study 2: The effects of supplementation with CPSM on calf intake and growth of Jersey calves.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Dairy farming is one of the main divisions in the livestock production (Habib *et al.*, 2007). Replacement heifers are part of the future dairy farm herd and it is the second major expenditure of the dairy industry (Drackley *et al.*, 2004). They should be managed well to substitute the aged and expensive cows. Dairy farmers have a challenge of high calf death rate and are unable to nurture replacement heifers (Razzaque *et al.*, 2009a). The losses of young calves were due to lack of proper medication (Postema *et al.*, 1987), inadequate management practices and high load of pathogens (Razzaque *et al.*, 2009a). The financial losses due to morbidity and death of calves were \$ 62.50 (US) per died calf (Razzaque *et al.*, 2009b). Thus, farmers try to optimise production and reproductive performance by applying new management procedures including feeding colostrum on time, nutrition, feeding and pen housing.

Many additives are added into the diets, administered orally or injected during pre-weaning period to provide noble groundwork for healthy and cost-effective heifers (Huyghebaert *et al.*, 2010; Phillips *et al.*, 2004). A number of feed additives affect aspect such as immunity and nutrition in cattle (Chaucheyras-Durand, & Durand, 2009). As such various additives are investigated nutritionally and therapeutically to enhance the livestock production (Huyghebaert, *et al.*, 2010).

2.2. Feeding management of dairy calves

2.2.1. Feeding colostrum

Neonatal calves are born agammaglobulinemic (Tizard, 1996; Quigley & Drewry, 1998; Weaver *et al.*, 2000). Neonatal calves are born without health resistance mechanism. Therefore, they totally rely on adequate absorption of maternal colostrum derived from Ig and essential for establishing passive immunity for its early diseases protection (Tizard, 1996; Weaver *et al.*, 2000). In mammalian animals placenta interferes with the transmission of Ig from the mother to the foetus. Therefore, the

intake of colostrum by the new born of these species has a fundamental role in acquiring immunity (Argüello *et al.*, 2004).

Colostrum is a combination of lacteal secretions and ingredients of blood serum that gather in the mammary glands and are aggregated via milk feeding of the earliest-lactating cow (Schoombee, 2011). Immunoglobulin is broken into five groups consisting IgM, IgA, IgD, IgG, and IgE where IgG, IgA and IgM are the major classes (Muller & Ellinger, 1981; Butler, 1983; Anderson *et al.*, 1985). These major classes differ in their concentrations, structures and functions. The IgG is produced in higher quantities than IgM (Anderson *et al.*, 1985). However, they are both important in the initial exposure to antigens and functioning in destroying pathogens. The IgA is insufficient to protect against intestinal pathogens and would interfere with rumen flora development (Muller & Ellinger, 1981; Anderson *et al.*, 1985). The consumption of optimal good colostrum aids in the physiological and development functions of the gut plus influencing the calf's nutritional condition and metabolic processes (Lee *et al.*, 1995; Guilloteau *et al.*, 1997; Bühler *et al.*, 1998). Colostrum tends to flush the digestive tract and retarding *Escherichia coli* bacteria from multiplying and migrating into rumen and abomasum where early death can be caused by a high concentration of microorganisms (Clapp, 1981). The calf's intestines are highly efficient at absorbing Ig at an early age of life (Anderson *et al.*, 1985).

Colostrum and transitional milk offer complete nutrients important for the survival of the neonate (Piccione *et al.*, 2009). Colostrum is rich in peptides, fats, fat-soluble, minerals, vitamins, and variety of enzymes (Grosvenor *et al.*, 1993). In addition, it comprises high concentrations of bioactive constituents such as lactoferrin, hormones, growth factors, lysozyme and lactoperoxidase. Transitional milk is produced on the second or third day after parturition and its composition is different from colostrum (Table 2.1).

Table 2.1: Compositional changes of colostrum over the first three milkings compared to transitional milk (Foley & Otterby, 1978).

Components	Milking after calving			Whole milk
	Colostrum	Transitional milk		
	Day 1	Day 2	Day 3	
Specific gravity (g/ml)	1.06	1.04	1.03	1.03
Solids (%)	23.9	17.9	14.1	12.9
Protein (%)	14.0	8.4	5.1	3.1
Fats (%)	6.7	5.4	3.9	3.5
Lactose (%)	2.7	3.9	4.4	5.0
Ig (%)	6.0	4.2	2.4	0.1
IgG g/L	48.0	25.0	15.0	0.6
Casein (%)	4.8	4.3	3.8	2.5

2.2.1.1 Passive immunity

In mammalian animals the placenta prevents transmission of Ig from the mother to the foetus (Argüello *et al.*, 2004; Weaver *et al.*, 2000). Passive immunity is the transfer of Ig from the mother to the new born and it is for protection from infectious sicknesses. Calves that do not obtain sufficient quantity of high quality colostrum early in life are prone to sicknesses, reduced growth performance and mortality (Donovan *et al.*, 1998; Virtala *et al.*, 1999; Tyler *et al.*, 1999; Weaver *et al.*, 2000). In the first 24 hours of life it is important for calves to obtain more than 10 mg/ml of serum Ig concentration (Schoombee, 2011). Calves will kick start to produce their own Ig nearly 10 days after partum and reach average serum levels at 8 weeks of age (Corbett, 1991; Hein, 1994).

2.2.1.2. The incidence of failure of passive transfer

The interaction factors (amount, quality, and timing of colostrum intake) influenced the pattern and rate of colostrum Ig absorption, to safeguard quality of protection against sicknesses (Weaver *et al.*, 2000). Furthermore, an incorrect timing, amount and quality of colostrum intake might results in decreased concentration of Ig circulating in the

blood (Gay, 1983). This condition is known as failure of passive transfer (FPT) and occurs when a calf's blood-serum concentration of IgG is less than 10 mg/ml (Schoombee, 2011).

Calves with failure of passive transfer (FPT) are at high risk of neonatal and pre-weaning sickness and death compared to calves with acceptable passive transfer (Wittum & Perina, 1995). The calves that received serum Ig below 10 mg/ml are more prone to diseases such as diarrhoea and pneumonia (McGuirk & Ruegg, 2011) and mortality (Schoombee, 2011).

2.2.2. Nutrient requirement and calves' growth

Growth is the basic processes that takes place in the life of animals and influenced by factors such as nutrition, genetic potential and environment (Krpalkova *et al.*, 2014). Growth in young calves before weaning occurs in the skeleton and muscle systems (Kertz *et al.*, 1998). Growth is the accumulation of new body tissue. Therefore, tissue growth is largely a function of protein deposition in bone and muscle, with corresponding mineralization of the protein matrix in bone. Rates of growth expressed as the percentage increase of body size are the highest at birth and decline steadily thereafter (Kertz *et al.*, 1998). Calves nutrition thus enters on provision of adequate energy and protein, while ensuring that all the required minerals and vitamins are consumed adequately (NRC, 2001).

The metabolisable energy and digestible protein for growth are provided to support the rate of body weight gain and body weight (NRC, 2001). For maximum and fast growth, calves need to consume more milk or milk replacer (Jasper & Weary, 2002) and dry feeds (Khan *et al.*, 2011). Calves respond to greater intake of milk or milk replacer by body weight gain (Uys *et al.*, 2011; Jasper & Weary, 2002).

Energy requirements for calves less than 99.8 kg body weight are established in units of metabolisable energy (NRC, 2001). Therefore, it is determined by subtracting losses of energy in faeces, digestive gasses (methane), and urine from digestible energy. The loss of energy in methane is insignificant in calves due to lower fat content in most milk replacer than whole milk (Holmes & Davey, 1976) and less metabolisable energy

per unit of solids (19.3 - 19.7 MJ/kg). Like energy, protein is required for both maintenance and growth. Unlike energy, however, protein requirements for maintenance are negligible and not believed to be substantially altered by cold or heat stress (Holmes & Davey, 1976). The average of 188 g of protein are deposited for every kg of body weight gain in calves to determine the rate of growth, which would require approximately 265 g of crude protein intake (NRC, 2001). Early nutrition provides optimal energy and protein, while ensuring that all required minerals and vitamins are consumed in adequate amounts and ratios to overall energy intake (NRC, 2001).

2.2.3. Feeding solid feed

The transition from pre-ruminant to a functioning ruminant requires consumption of starter dry feed (Khan *et al.*, 2011). This promotes rumen development and achieves optimal growth (Quigley, 2001). Calves should be provided with water and a palatable nutritious starter from the first week of age until they are weaned (Drackley, 2008). The provision of sufficient water influences the growth of bacteria and ruminal development (Quigley, 2001).

The development epithelium of the rumen is controlled by chemical but not physical means (Quigley, 2001). The primary stimulus for the ruminal epithelium development to ruminal papillae is the fermentation of dry feeds to volatile fatty acids (VFAs) (Heinrichs & Lesmeister, 2005). If calves are offered only milk, no microbial fermentation occurs in the rumen, thus the production of ketones at this time is very small (Baldwin *et al.*, 2004). The existence of beta-hydroxybutyric acid (BHBA) in the blood of juvenile calves is an indication of rumen development, such that BHBA is produced from butyrate by the rumen epithelium (Coverdale *et al.*, 2004; Khan *et al.*, 2011). An increase in blood BHBA with age is thought to indicate a shift from a pre-ruminant to a functioning ruminant (Khan *et al.*, 2011). Butyrate is the most stimulatory of the VFAs produced by the rumen with regards to development of epithelium (Tamate *et al.*, 1962). There is over-institution of ruminal papillae when VFAs levels are high. Sometimes ruminal papillae may clump together during this process to reduce available surface area for absorption (Quigley, 1997). In addition, some

movements are essential to control the papillae free from keratin layers that can inhibit VFA absorption.

2.2.4. Feeding milk or milk replacer

In dairy farming, calves are removed from their dams within first 24 hours of age (Rushen *et al.*, 2008). Calves can be fed milk or milk replacer twice a day to reach approximately 10 % of their birth body weight. Calves can also be fed high levels of milk to enhance body weight and frame size growth (Yavuz *et al.*, 2015).

Restricted milk feeding programmes is achieved by encouraging early intake of dry feeds to stimulate the development of rumen and to achieve reasonable animal performance using less milk (Khan *et al.*, 2011). There is a concern in the industry that calves fed high levels of milk may show increased occurrence of diarrhoea (Diaz *et al.*, 2001). There is disagreement with respect to the incidents and incidence of diarrhoea when calves consume increased levels of milk; in some cases. This may be confused with higher faecal score. There is a higher occurrence of diarrhoea in calves supplied with higher levels of milk or milk replacers, compared to calves given restricted milk (Quigley *et al.*, 2006). In contrast it was observed that there was no difference (Jasper & Weary, 2002).

2.2.5. Management of the dairy calf for health

2.2.5.1. Hygiene and calf health

The calves' environs should be dry, clean, adequately ventilated and comfortable (Maunsell & Donovan, 2008). Thus, hazard analysis critical control point method was developed to reduce calfhood diseases by decreasing the environmental pathogen load at calf rearing area (Cullor 1995; Noordhuizen & Metz, 2005). The control of pathogen load in the environment of calves reduces the occurrence of infections and diseases plus the potential of pathogens to infect humans (McGuirk, 2008).

Bio-security plans regulate and prevent the introduction and spread of infectious agents (Noordhuizen & Da-Silva, 2009). Bio-security plans are sometimes referred to as health management strategies and consist of formal ailment risk identification and assessment on farms (Stanković *et al.*, 2016). These plans make proper use of various protocols. For example, entrance procedures for animal farmers, a protocol on general hygiene, on animal treatment and on disease diagnostics.

2.2.5.2. Calf housing and health

Calf housing can have positive or negative impacts on the health status and performance of animals (McGuirk, 2008). In a pre-weaning home calves are provided with dry shelter and clean bedding (Risco & Melendez, 2011). There is a high risk of infections in group housing, because calves are at close range with each other (Steenkamer, 1982). Individual housing during the pre-weaning period is a popular management to control the horizontal transmission of infectious diseases (Fourichon *et al.*, 1997; Gulliksen *et al.*, 2009) but it is expensive. It is not easy to keep operative sanitation, nutrition management and control diseases of large group housing (Gulliksen *et al.*, 2009). Therefore, calves are prone to high levels of pathogens when housed in groups, resulting in higher rates of sickness and death.

Calves in large groups are at high risk of diarrhoea and its rate was positively correlated to the herd size (Frank & Kaneene, 1993). Furthermore, calves in large groups experienced increased risk of respiratory diseases and diarrhoea compared to single housing or small group according to Lundborg *et al.*, (2005) and Frank & Kaneene, (1993), respectively. The detection of illnesses and treatment might be delayed and more costly in large groups (Van Putten, 1982).

2.2.5.3. Common diseases in calves

Calves are generally susceptible to infections from pathogens found in the calving area before and during calving (Donovan *et al.*, 1998; McGuirk & Ruegg, 2011). During the first 24 hours post-partum, the gut absorbs large IgG molecules from colostrum to warrant passive immunity (Anderson *et al.*, 1985). This absorption of IgG is more efficient at destroying pathogens and important in the initial exposure to antigens.

Calves that fail to absorb high quality of colostrum are more susceptible to be infected by the agents causing diseases (McGuirk & Ruegg, 2011). Common syndromes that are associated with young calves are diarrhoea and respiratory diseases (McGuirk & Ruegg, 2011). These syndromes have major impacts on the livestock industry worldwide resulting in economic losses thus ranked as the most important contagious diseases of cattle and buffaloes (Bartels *et al.*, 2010).

a) Calf diarrhoea

Calf diarrhoea is a multifactorial sickness that can result in serious economic and animal wellbeing implications in dairy and beef herd (Muktar *et al.*, 2015). It is one of common illness reported in calves in first three months of age (Svensson *et al.*, 2003). Bacteria, viruses, protozoa and/or parasites are associated with calf diarrhoea (Bhat *et al.*, 2013; Singla *et al.*, 2013). Some agents implicated to calf diarrhoea are *Rotavirus*, *Coronavirus*, *Viral diarrhoea virus*, *Salmonella species*, *Escherichia coli*, *Clostridium species* and *Cryptosporidium species* (Muktar *et al.*, 2015). However, stress, insufficient intake of colostrum, poor sanitation, and inadequate ventilation, overcrowding and cold might cause calf diarrhoea (Lance *et al.*, 1992; Muktar *et al.*, 2015). The disease progress rapidly resulting in death within 24 hours. Dry cow vaccination, satisfactory bio-security and optimal intake of quality colostrum can avoid the diseases (McGuirk & Ruegg, 2011).

b) Bovine respiratory diseases

Bovine respiratory disease (BRD) appears to be a result of the cocktail of many viral and bacterial pathogens, stress and immunity of the individual animal (Wikse & Baker, 1996). The BRD is one of the health challenges worldwide, resulting in monetary losses and mortality in dairy and beef herds (Woolums *et al.*, 2005). This resulted in increased antibiotic usage, decreased growth performance, reduced animal wellbeing, and production efficiency (Holland *et al.*, 2010). The clinical symptoms are rectal temperature of more than 40 °C, lethargy, decreased appetite, runny nose, increased respiratory rate, coughing, separation from the herd and gaunt appearance (Powell, 2010). The primary viral pathogens associated with BRD are bovine rhinotracheitis, parainfluenza-3, bovine respiratory syncytial and bovine diarrhoea virus (Faber *et al.*, 2000). Immune-suppression from stress and viral infections allow bacteria in the

respiratory tract to overcome immune response and multiply in the nasopharynx and lungs causing pneumonia (Hodgson *et al.*, 2005).

2.2.6. Feed additives in dairy calves feeding

2.2.6.1. Antimicrobial feed additives

Antibiotic compounds contain antimicrobial activity that can be used orally, parentally or topically (Phillips *et al.*, 2004). They were used in human and veterinary, for various purposes to prevent or to treat diseases and as growth promoters. Ionophores are lasalocid, monensin, narasin salinomycin, tetranasin, while and non-ionophores are avoparcin, flavomycin, tylosin, virginiamycin, and bacitracin (Nagaraja *et al.*, 1997; Chesworth *et al.*, 2012). Both ionophores and non-ionophores, are antibiotic growth promoters that have effects on gram-positive bacteria. Some are used to advance the health and welfare of animals, which are metaphylaxis and prophylactics (Huyghebaert *et al.*, 2010). Metaphylaxis antimicrobial treatments are aimed to treat sick animals and prevent diseases (Mc Ewen & Fedorka-Cray, 2002). Prophylactics are mostly provided during high-risk periods for infectious diseases in animals as well as antimicrobial growth promoters (Huyghebaert *et al.*, 2010).

The European Commission phased out the use of antimicrobial growth promoters as veterinary medicine and feed supplements and ultimately ban from trading (Pugh, 2002). Furthermore, this was influenced by the emergence of pathogens resistant to antibiotics. The use of antibiotics in animals has an insightful effect on animal health and wellbeing (Rigobelo & De Ávila, 2012).

2.2.6.2. Phytogetic feeds

Phytogenics are derived from herbs, spices are non-antibiotic growth promoters used as feed supplements and for the preservation of food (Windisch *et al.*, 2008) as are extracts or essential oils (Hong *et al.*, 2012). These products are utilised as natural alternatives to maintain animal health and performance (Steiner & Syed, 2015). Some plant extracts have antimicrobial, antioxidant, antiviral, anticoccidial, and fungicidal properties related to their lipophylic characters (Giannenas *et al.*, 2003). Phytogetic agents from oregano, cinnamon and thyme have broad antimicrobial actions against

pathogenic bacteria (Applegate *et al.*, 2010). The phytochemicals stimulate digestive secretions including saliva and endogenous enzymes (Williams & Losa, 2001). Certain phytochemicals (saponin) are likely to lessen ammonia emission of animals by inhibiting the urease activity that converts urea into ammonia and CO₂ (Veit *et al.*, 2011). The milk blend with plant extracts was formulated to increase palatability, digestion and to improve zootechnical parameters to maintain a better intestinal health and reduce diarrhoea (Steiner & Syed., 2015).

2.2.6.3. Live microorganisms

Probiotics are live microorganisms that may affect the host animal to improve the balance of the gut microflora (Musa & Seri, 2009) amounts. When appropriate amount of probiotics are used they improve the health status of the host (Chaucheyras-Durand & Durand, 2009). They improved the growth of livestock, efficacy of feed digestion and quantity plus quality of meat, milk and eggs (Musa & Seri, 2009). Most of the probiotic microorganisms are part of lactic acid producing bacteria such as *Aerococcus*, *Atopobium*, *Bifidobacterium*, *Brochothrix*, *Carnobacterium*, *Enterococcus*, *Lactobacillus* and *Weissella* (Ferreira & Viljoen, 2003). Bacterial probiotics are effective in chickens, pigs and pre-ruminant calves (Musa & Seri, 2009). The yeast and fungal probiotics such as *S. cerevisiae* and *Aspergillus oryzae* improved adult ruminants (Musa & Seri, 2009). The administration of probiotic strains in combination might improve health benefits compared to single strain (Collado *et al.*, 2007).

2.3. *Carica papaya* seed as additive in animal production

2.3.1 Chemical composition of *Carica papaya* seed

The papaya tree belongs to the family Caricaceae with four genera in the world: *Carica papaya* Linn, *Carica cauliflora* Jacq, *Carica pubescens* Lenne and *Carica quercifolia* Benth (Anibijuwon & Udeze, 2009). *Carica papaya* Linn is one of the most cultivated of the Caricaceae family and used for its food, nutrition and medicinal benefits (Krishna *et al.*, 2008). The leaves, bark, fruit, flowers, seed, latex, and roots are used for medicinal and various other purposes (Jaiswal *et al.*, 2010).

The seeds are black, tuberculous and contained in a translucent aril (Rupa & Jayanta, 2013). The seeds of *C. papaya* Linn are characterised by fatty acid, crude protein and

fibre, and oil (Krishna *et al.*, 2008). The moisture content is 4.93 - 6.2 g/100g, protein 27.8 - 30.5 g/100g, carbohydrate 6.2 - 14.6 g/100g, lipids 28.3 - 60.1 g/100g and fibre 22.6 - 60.7 g/100g (Marfo *et al.*, 1986; Dakare *et al.*, 2011). The chemical fatty acid composition of papaya seeds is arachidonic acid, behenic acid, myristic acid, palmitic acid, stearic acid, and unsaturated fatty acids (Puangsri *et al.*, 2005). In addition, phospholipids, carpaine (an alkaloid), benzyl isothiocyanate, benzyl glucosinolate, glucopeolin (Rossetto *et al.*, 2008), hentriacontane, sitosterol, caricin and myosine enzyme were identified (Rastogi & Mehrotra, 1993). *Carica papaya* contains many biochemically active composites (Anibijuwon & Udeze, 2009). The important composites that aid in digestion are chymopapain and papain (a proteolytic enzyme) (Anibijuwon & Udeze, 2009) and improves digestion of food protein with various levels of pH (Krishna *et al.*, 2008).

Papaya seeds contain 50; 220; 130; 17 340; 520; 3; 340; 110 and 10 250 ug/g of copper, magnesium, iron, calcium, sulphur, manganese, potassium, sodium and phosphorus, respectively (Marfo *et al.*, 1986). The mineral composition is lower compared to other plant seeds (Afolabi *et al.*, 1985). The chemical composition makes the papaya seeds a rich source of nutrients, and has toxicants such as glucosinolates in the seeds and extracts (Marfo *et al.*, 1986).

2.3.2. Antibacterial property of *Carica papaya* seed

Papaya plants have anthelmintic properties that contain plant secondary metabolite compounds that are directly active against parasites (Githiori *et al.*, 2006). The various preparations of papaya seeds can kill helminths *in-vitro* and *in-vivo* (Krishnakumari & Majumder, 1960; Kermanshai *et al.*, 2001). The seed extracts contain anthelmintic action against *Ascaris lumbricoides* (Dar *et al.*, 1965) and *Caenorhabditis elegans* (Kermanshai *et al.*, 2001) due to benzyl isothiocyanate (BITC). The seeds are used as carminative, emmenagogue, stomachic, antidyseptic, diuretics, vermifuge, antiasthmatic treatment of ringworms and psoriasis as well as a cure for enlargement of liver and spleen (Afolayan, 2003; Krishna *et al.*, 2008; Rupa & Jayanta, 2013).

2.3.2.1 Secondary metabolites of *Carica papaya* seed

Papaya seeds and their extracts contain secondary metabolites (phytochemical compounds) such as benzyl isothiocyanate, carpaine (an alkaloid) and papain, chymopapain (enzymes) (Kermanshai *et al.*, 2001; Ikram *et al.*, 2015). Furthermore, tannins (Marfo *et al.*, 1986), steroids, cardioactive glycosides, triterpenoids and flavonoids were present in the papaya seeds (Pinto *et al.*, 2015; Ikram *et al.*, 2015). These compounds exhibit the anthelmintic and antimicrobial activities of papaya seeds (Krishnakumari & Majumder, 1960). Consumption of tannins has been associated with increased milk production, ovulation rate, decreasing bloat and internal parasite load in ruminants (Min *et al.*, 2003). This is related to tannins improving essential amino acids absorption from the small intestine. Tannins in papaya seed oil control bacteria, viruses yeast and protozoa (Davidson & Branden, 1981). In addition, essential oil was used to improve growth performance (Hong *et al.*, 2012).

The BITC is a derivative of the enzyme myrosinase (thioglucosidase) on benzyl glucosinolate action (Bennett *et al.*, 1997), which occurs during the crushing of the seeds (Tang, 1973). The BITC seems to be the principal or sole anthelmintic element in the papaya seeds (Kermanshai *et al.*, 2001). Treatment of ruminants with papaya seeds has found to be harmful to intestinal microorganisms and killing nematodes (Emeruna, 1982). Chymopapain and papain are identified as important compounds, which are used as medicine and aid in digestion (Anibijuwon & Udeze 2009).

2.3.3. Feeding *Carica papaya* Linn to ruminant

The use of exogenous enzymes as an alternate natural product in animal nutrition has improved animal production, reduce the sicknesses and death in intensive farming systems (El Nenei *et al.*, 2015). The enzymes used as additives improve the performance by reducing the thickness of intestinal contents and enhancing the diet nutritive value (Barletta, 2011). Papaya latex can be used as an alternative growth promoter and/or enhance the immune system (El Kholy *et al.*, 2008; El Nenei *et al.*, 2015). In addition, it may cause absorption of amino acids because of cysteine proteinases (digestive enzymes) (El Moussaoui *et al.*, 2001; Azarkan *et al.*, 2003) due to the presence of antibacterial properties. Papaya latex improved digestibility of nutrients, absorption and intestinal morphology characteristics, this reaction can lead

to increased feed utilization and improved performance (El Neneiy *et al.*, 2015). There is limited evidence on administration of papaya seeds to ruminant feed or milk as a feed additive.

CHAPTER 3

The effects of *Carica papaya* seed (*Linn*) meal on faecal pathogens population in dairy calves and on its influence on calf performance

3.1. Introduction

Pathogens are identified as the main cause of diseases that have deleterious effects such as diarrhoea, weight loss, renal failure and low performance in animals (Garner & Ware, 2006). Several pathogens, singular or in combination, are etiologic agents of sickness in calves (Barrington *et al.*, 2002). Most of these agents are transmitted through faecal-oral of susceptible animals. The calf's exposure to pathogens, the weather conditions, poor hygiene, and the nutritional and immunological condition of calves contribute to the transmission (Barrington *et al.*, 2002). These factors resulted in respiratory diseases and diarrhoea. Furthermore, these incidences are high in intensive farming systems, where exposure to pathogens is greater (Callaway *et al.*, 2002).

Enteropathogenic bacteria for example *S. aureus*, *E. coli* and *Salmonella species* are identified as the main cause of intestinal diseases (Garner & Ware., 2006). Some hundreds of strains of *E. coli* are harmless and live in the intestines (O'Brien *et al.*, 1984; Garner & Ware, 2006). However, some strains of *E. coli* produce large quantities of toxins. These toxins can cause severe distress in the small intestine and often result in damaging the intestinal epithelium and cause extreme cases of diarrhoea. These conditions are related to reduced body weight gain and increased death rates in cattle production (Virtala *et al.*, 1996). The objective of this study is to ascertain the effects of supplementation with *Carica papaya* seed (*Linn*) meal on faecal pathogens population in dairy calves and on its influence on calf performance.

3.2. Materials and methods

The experiment was carried out at Agricultural Research Council-Animal Production Institute (ARC-API) dairy section in Irene, South Africa. The animal ethic committees of both the Agricultural Research Council-Animal Production Institute, (APIEC15/038) and University of South Africa, (2015/CAES/114) approved the research proposal and

use of animals. Animal care was consistent with the guide for care and use of animals in ARC policies and procedures.

3.2.1 Seed meal preparation

The *Carica papaya Linn* fruits were obtained from the Johannesburg fruit and vegetable market. The fruits were cut longitudinally into two halves. The seeds were removed by hand and then weighed before dipped in water overnight, to allow the sarcotesta membrane to swell for easier removal. The sarcotesta membrane was removed by squeezing the seeds between fingers. Seeds were air dried for three days in a forced draft oven at 60 °C and thereafter ground using a blender, and stored for further use.



Figure 3.1: The cross section of *Carica papaya Linn* fruit.

3.2.2. Animals and diets

Six Holstein female calves aged 6 (\pm 2) days and weighing 38.7 (\pm 4.7) kg body weight (BW) were assigned to two treatments (control and *Carica papaya* seed meal (CPSM)) for 14 days. Calves were housed in a single pen and were fed restricted milk (3 L at 08:00 and 3 L at 14:00). Calves were provided with calf starter feed in the form of pellet

at *ad lib* and had access to fresh water. The calves fed CPSM received 5 g CPSM on day 8 and 9 of the experimental period (14 days). The chemical composition of the calf starter pellet is shown in Table 3.1.

Table 3.1: Chemical composition of the calf starter feed.

Nutrients	Amount
Dry matter, g/kg	892
Crude protein, g/kg of DM	181
Fat, g/kg of DM	35
NDF, g/kg of DM	219
ME, MJ/kg	14.3
Ca, g/kg of DM	8.1
P, g/kg of DM	5.1

¹Contained a premix that supplied per kg: 3000 IU Vitamin D3, 15000 IU Vitamin A., 30 mg Vitamin. E, 125 mg niacin, 0.15 mg Co, 10 mg Cu, 50 mg Fe, 50 mg Mn, 180 mg P, 0.15 mg Se, 50 mg Zn, 0.8 mg I, and 50 mg antioxidant.

²Metabolisable energy calculated according to NRC (2001).

3.2.3. Feed and milk intake

Calf feed intake was determined as the difference between feed offered and feed refusal. Intake of milk was recorded every time the milk was offered to calves. Dry feed and milk nutrient intakes were calculated from feed and milk composition.

3.3. Measurements and sample collection

Fresh faeces were sampled from the rectum of each calf on day 7 (the day before calves were fed CPSM) and day 10 (two days after receiving CPSM). The faecal samples were immediately taken to the laboratory for the enumeration of coliforms, *E. coli* and *Enterobacteriaceae*. Calves were also weighed on day 7 and 14 of the experimental period. Intake of feed was measured daily from feed offered and refusals and milk nutrients were calculated from feed composition. The enumeration of coliforms was done according to ISO Standards 4832 (2006), and *Enterobacteriaceae* according to ISO 21528-1 (2004). For chemical milk analyses, composite of daily milk samples from the bulk tank were collected in the morning and evening on monthly basis to determine fat, crude protein and lactose at Lacto Lab (Pty) (Irene).

3.3.1. Feed sample analyses

Calf starter diet was sampled and analysed for DM by oven drying at 60 °C for 48 hours. Dried samples were ground and analysed for crude protein (CP) and ether extract according to AOAC (2000). Calcium (Ca) and potassium (K) were determined according to Giron, (1973). Phosphorus (P) was assayed according to AOAC (2000) The NDF was determined according to Van Soest *et al.*, (1991).

3.4. Statistical analyses

Data were statistically compared using independent two t-tests, recommended for small sample size (De Winter, 2013). Statistical analyses were performed using SAS (2014). The model was:

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - m}{\sqrt{s^2 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}}$$

Where:

m = difference between means,

x_1 = sample mean for the first sample,

x_2 = sample mean for the second sample,

n_1 = sample size for the first sample, and

n_2 = sample size for the second sample.

The pooled sample standard deviation was calculated as:

$$s^2 = [((n_1-1) s_1^2 + (n_2-1) s_2^2) / (n_1+n_2-2)]$$

Significance was reported at $p < 0.05$ and tendencies at $p < 0.10$.

3.5. Results

The effects of supplementing calves with CPSM on intake and faecal pathogens are in Table 3.2 and 3.3 respectively.

Table 3.2: The effects of CPSM on feed and nutrient intake

Parameters	Treatments		SEM	p- values
	Control	CPSM		
Total DMI, g/d	850	1000	0.17	0.04
Total CPI, g/d	230	250	0.03	0.04
Total fat, g/d	230	230	0.007	0.08
MEI, MJ/d	4.19	4.92	0.005	0.17

SEM: Standard error of mean; CPSM: *Carica papaya* seed (*Linn*) meal; DMI: Dry matter intake; CPI, Crude protein intake; MEI: Metabolisable energy intake

Intake of dry matter and CP intake were higher ($p < 0.05$), and fat intake tended to be higher ($p = 0.08$) when calves were fed CPSM compare to control calves. The MEI did not differ between the CPSM and control calves.

Table 3.3: The effects of CPSM on faecal coliform counts (*Escherichia coli* and *Enterobacteriaceae*)

Parameters (CFU)	Treatments		SEM	p- values
	Control	CPSM		
Total coliforms, cfu				
Day 7	2.4×10^8	1.5×10^7	2.8×10^7	0.02
Day 10	2.6×10^8	1.7×10^6	2.9×10^7	< 0.001
<i>Escherichia coli</i>, cfu				
Day 7	2.0×10^8	1.3×10^8	5.6×10^7	0.24
Day 10	2.2×10^8	8.7×10^6	5.8×10^7	0.027
<i>Enterobacteriaceae</i>,				
Day 7	1.3×10^9	4.3×10^8	4.0×10^8	0.15
Day 10	1.3×10^9	4.8×10^7	4.1×10^8	0.06

SEM: Standard error of mean; cfu: Colony forming unit; CPSM: *Carica papaya* seed (*Linn*) meal

The total coliforms were lower in calves fed CPSM compared to control fed calves on day 7 and day 10. The *E. coli* was significantly lower ($p = 0.03$) in calves fed CPSM only on day 10. The *Enterobacteriaceae* count tended to be lower in CPSM fed calves compared to control calves.

The distribution of the three pathogens groups are presented in Figures 3.2; 3.3 and 3.4.

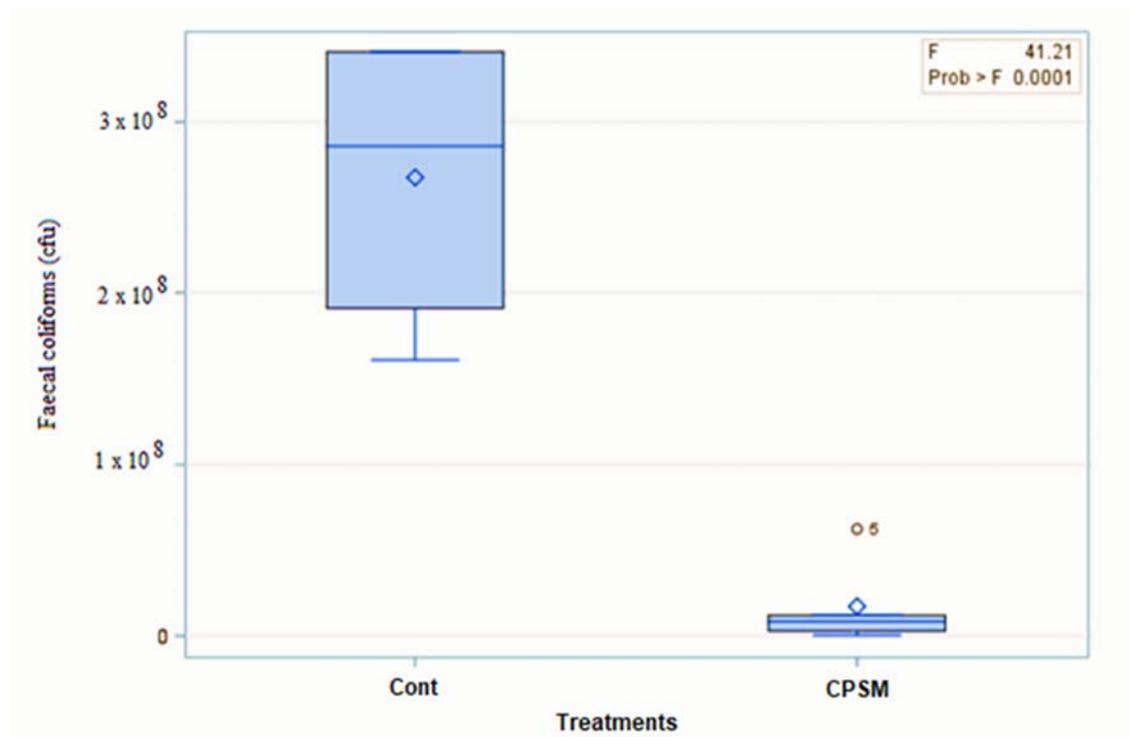


Figure 3.2: Distribution of coliforms in calf faecal samples as affected by supplementation with CPSM.

There was a net separation in the distribution of faecal coliforms count for control fed calves and calves fed CPSM. Coliforms in control fed calves ranged from 2.4×10^8 to 2.6×10^8 cfu, and in CPSM-fed calves it ranges from 1.5×10^7 to 1.7×10^6 cfu.

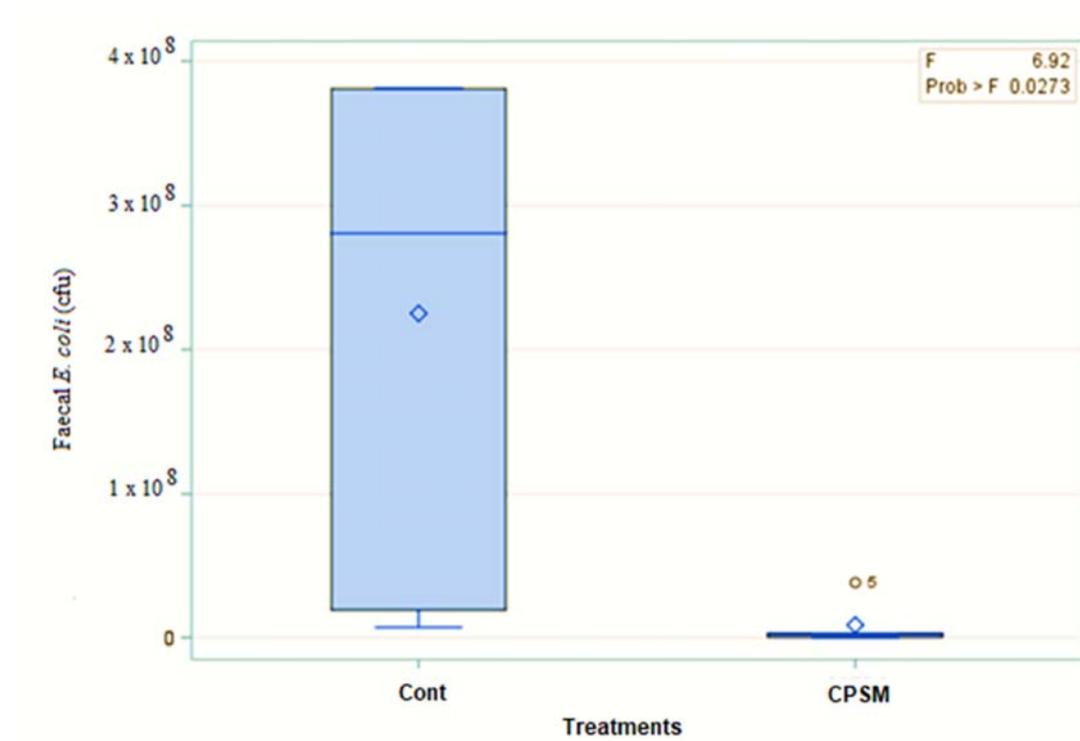


Figure 3.3: Distribution of *Escherichia coli* in calf faecal samples as affected by supplementation with CPSM.

There was also a difference in the distribution of faecal *E. coli* counts between control and CPSM fed calves. The *E. coli* in control fed calves ranged from 2.0×10^8 to 2.2×10^8 cfu and in CPSM fed calves it ranged from 1.3×10^8 to 8.7×10^6 cfu.

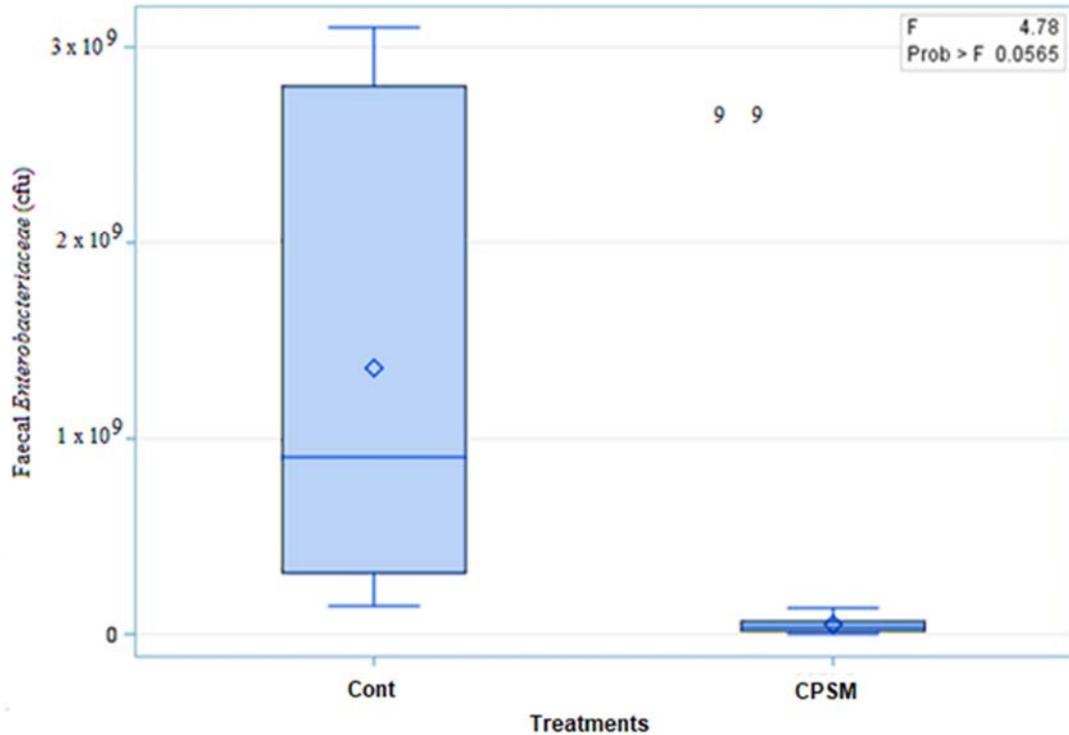


Figure 3.4: Distribution of *Enterobacteriaceae* in calf faecal samples as affected by supplementation with *Carica papaya* seed (*Linn*) meal (CPSM).

The faecal *Enterobacteriaceae* ranged from 1.3×10^9 to 1.3×10^9 cfu in control fed calves, and 4.3×10^8 to 4.8×10^7 cfu in CPSM fed calves, also showing a net distribution difference. The effects of *Carica papaya* seed (*Linn*) meal on faecal pathogens was calculated and presented in Figure 3.5.

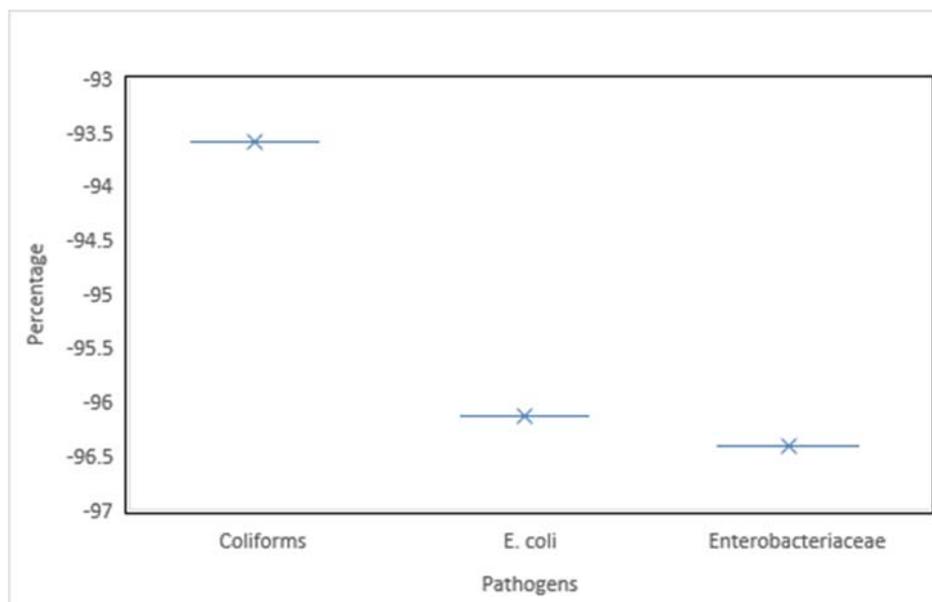


Figure 3.5: The effect of *Carica papaya seed (Linn)* meal on percentage faecal pathogen reduction.

Supplementation of CPSM to calves resulted in 93.6; 96.1 and 96.4 % reduction ($p < 0.05$) of faecal coliforms, *E. coli* and *Enterobacteriaceae*, respectively.

3.6. Discussion

3.6.1 Dry matter and crude protein intake

In the current study, calves fed CPSM consumed more DM and CP; 1000 g/d and 250 g/d respectively than control fed calves. Farrag *et al.*, (2013) reported improved nutrient intake when papaya seed powder was added in the feed of calves. In addition, calves fed papaya seed powder consumed more feed and consequently more nutrients than control fed calves. Papaya seeds contain a proteolytic enzyme (papain), which improves digestion of dietary protein, leading to increased intake (Krishna *et al.*, 2008). The addition of papain to some of the mixture of forages enhanced the availability of protein thus reduced the cost of the forage and exploited sources of protein (Wong *et al.*, 1996). This happened at acid, alkaline as well as neutral medium. In broilers fed crude papain extract from the papaya plant an increase in digestibility and the ability of nutrients absorption was observed (Rumokoy *et al.*, 2016). No differences in fat and ME intake was observed in the present study. When evaluating papain extract in chickens, Rumokoy *et al.*, (2016) observed a tendency to increase

intake of ME, which suggested a different mode of action in birds or it acts more extensively and thereby affecting feed nutrient utilisation. In addition to papain, the presence, and the ability of cysteine proteinases (digestive enzymes) in papaya latex may also have contributed to the increased feed intake. This may be caused by absorption of amino acids (El Moussaoui *et al.*, 2001; Azarkan *et al.*, 2003). In addition, improved carbohydrate digestibility in rabbits was observed by Sequera *et al.* (2000) though they are monogastric animals.

3.6.2 Enteric pathogens

Gut microbial balance is the most important factor that promotes a good health status in calves (Tsuruta *et al.*, 2009). It was reported that reducing the load of opportunistic pathogens protect the gastrointestinal tract of an animal, and lowered the incidence of intestinal and respiratory diseases (Callaway *et al.*, 2002; Barrington *et al.*, 2002). Furthermore, gut microbial balance improved feed utilisation and animal performance. Plant extracts exhibit antibacterial activities against a wide range of pathogens and may be used as growth promoters in livestock as well as to control pathogens (Baladrin *et al.*, 1985; Githiori *et al.*, 2006). Infectious bacteria such as enteropathogens, *E. coli*, *Salmonella* and *Clostridium* species are main microorganisms that cause infectious diseases (diarrhoea and pneumonia or respiratory disease). The decrease in their numbers is an indication of a healthy gut.

In the present study, the total number coliforms, *E. coli* and *Enterobacteriaceae* were reduced by more than 90 % after supplementation with CPSM for two days. This can be attributed to the presence of secondary metabolites as suggested in other studies (Osato *et al.*, 1993; Githiori *et al.*, 2006). This is supported by (Panse & Paranjpe, 1943; Kermanshai *et al.*, 2001), who observed that papaya seeds contain bioactive compounds such as BITC, carpaine (an alkaloid) and carpasemine, which attributes to the antimicrobial and anthelmintic properties of the seeds (Osato *et al.*, 1993). The BITC is reported to be bacteriostatic against *B. subtilis*, *Enterobacter cloacae*, *E. coli*, *Salmonella typhi*, *S. aureus*, and *Proteus vulgaris* (Osato *et al.*, 1993). The anthelmintic properties of papaya plant against some parasites were attributed to the presence of secondary metabolites (Githiori *et al.*, 2006).

In the present study, the number of faecal coliforms, *E. coli* and *Enterobacteriaceae* were significantly reduced after 5 g of CPSM were added to the feed for two consecutive days. This indicated that *Carica papaya* seeds reduced the number of pathogens due to the presence of antibacterial properties.

3.7. Conclusion

The current study investigated the effects of CPSM on faecal bacteria in calves. The results of this study showed significant reduction of bacteria and increases DMI and CPI, supporting the hypothesis. This support the finding on effects of pathogenic feeds on animals and human to maintain health and performance as well as to inhibit pathogenic bacteria colonization of the digestive tract.

The development of many diseases occurred during the early life of calf involve increased in number of pathogenic bacteria. This resulted in high losses of calves. Thus, CPSM can be considered as an alternative to antibiotics due to its positive effects on inhibiting pathogens. Furthermore, effects of reducing faecal pathogens with CPSM suggested a healthy gut environment, consequently enhanced intake of dry matter. Eventually increased the utilization of feed nutrients and improved calf growth performance. Consequently, this improved animal health and production.

The recommendation is to evaluate the interaction among graded levels of CPSM and different periods for a long term on health and performance of calves.

CHAPTER 4

The effects of supplementation with *Carica papaya* seed (*Linn*) meal on feed intake and growth of Jersey calves.

4.1. Introduction

In the dairy industry calves are more susceptible to diseases early in life due to inadequate colostrum, and low feed intake resulting in a retarded growth rate (Davis & Drackley, 1999). Increased calf digestive disorders negatively affect health, nutrient absorption and consequently calf growth performance. It is vital to minimise the occurrence of gut infections in calves because when animals are sick during this stage their succeeding growth stages will be retarded eventually affecting the animal production (Rosmini *et al.*, 2004). Optimum pathogens in the gut results in good health, positively effects feed utilisation and growth (Tsuruta *et al.*, 2009). The diseases are identified as the main effect on financial viability in cattle farming due to high death, high cost of medication and poor performance of calf (Postema *et al.*, 1987).

Calves are fed antibiotics, probiotics and non-antimicrobial feed additives in feeds to reduce the occurrence of infectious sickness and to improve animal performance (Galvão *et al.*, 2005). *Carica papaya* seeds and their extracts might contain adequate antibacterial and anthelmintic properties that may be used for the treatment of the bacterial infections and as growth promoters (Baladrin *et al.*, 1985). The objective of this study is to determine the effects of supplementation with *C. papaya* seed (*Linn*) meal on feed intake, growth and structural body parameters of suckling Jersey calves

4.2. Materials and methods

The experiment was conducted at the calf-rearing section of the Bethel Agricultural College in Butterworth, Eastern Cape (South Africa). The Ethical approvals were obtained as mentioned in Chapter 3. The preparation of *C. papaya* (*Linn*) seeds meal is similar to the method explained in the first experiment (Chapter 3; Section 3.2.1). Animal care was consistent with the policies and procedures for the care and use of animals in ARC.

4.2.1. Animals and experimental design

Eighteen Jersey calves 22.2 ± 1.49 kg BW were blocked based on birth weight and sex. All calves were randomly assigned at birth to one of the three treatments (four females and two males per treatment group). Treatments were a control group (con) (not supplemented: control), a group that received 5 g/d (3.2×10^8 cfu) of *Lactobacillus acidophilus* (Lact) and a group that received 5 g/d of *Carica papaya* seed meal (CPSM). Immediately after birth, the calves were hand-fed 2 L of milk with colostrum from their dams within 4 hours after birth and another 2 L within 12 hours after birth. The same amount of milk from the dams was fed to calves for three consecutive days in the morning (08:00) and afternoon (14:00). *Carica papaya* seed meal (5 g) and *Lactobacillus acidophilus* (Lact) (5 g) were added to whole milk from day four and bottle-fed to calves only in the morning before suckle from the dams. All calves were allowed to suckle from the dams three times a day (at 08:00; 12:00 and 17:00). From day 4 a commercial calf starter in pellet form was added in their diets (Table 3.1; Section 3.2.2) and fresh water *ad-libitum*. Animals were closely monitored for clinical signs of diarrhoea or other metabolic problems.

4.2.2. Housing and calves' management

After three days of milk with colostrum from the dams (2 L at 08:00 and 2 L at 14:00), CPSM and Lact (Biorem, South Africa) were separately added to whole milk from day 4. Calves were bottle-fed before allowed to suckle from their respective dams three times a day (08:00; 12:00 and 17:00) for 30 minutes. A commercial calf starter feed and fresh water were available *ad libitum* starting from day 4 of age until weaning at 42 days of age. Calves were treated with Wound Sept-plus (Oberon Pharma) for three days after birth to prevent infections. All calves that showed signs of illness were separated from others and treated until recovered. Special care was taken to provide clean milk and water to the calves. Milk bottles and teats were washed and immersed in boiled water before milk feeding. After suckling from the dams, calves were moved back into their pens (roofed shelter). The pen floor was made of concrete, rubber mats and fodder hay functioned as bedding.

4.3 Parameters measured

4.3.1. Calf intake and body weight

Daily calf starter intake was determined from amount fed minus orts, and recorded. Calves were weighed at birth and every seventh day until weaning.

4.3.2 Growth parameters

Growth parameters: body weight (BW), heart girth (HG), hip width (HW), hip height (HH) and shoulder height (SH) were measured at birth and every seventh day thereafter to determine overall tissue and skeletal growth. For BW, a mechanical spring dial hoist scale with accuracy to the nearest 200g (<20 kg) or the nearest 500g (>50 kg) was used. Remaining growth measurements were done with a measuring tape to the nearest 5 mm.

- Shoulder height: the distance from the ground measured around the peak of the shoulder blades.
- Hip height: the distance from the ground directly over the point of the hip with the calf standing on level ground.
- Hip width: the widest point at the centre of the stifle.
- Heart girth: the distance around chest directly under the armpits.

4.4 Feed sample analyses

These analyses were conducted as mentioned in Chapter 3, Section 3.3.1.

4.5 Statistical analyses

Data were analysed as repeated measures using the PROC MIXED model of SAS (SAS Institute, 2014). Parameters were pooled on weekly basis for analyses. The statistical model included calf as a random effect, and experimental group and its interaction with time as a fixed effect. The statistical model used was:

$$Y_{cgt} = \mu + \alpha g + \beta t + (\alpha\beta)gt + \gamma(\alpha)cg + ecgt,$$

Where Y_{cgt} = an observation value for parameters measured from calf c from group g at time t ;

μ = overall mean for the population;

αg = fixed effect of group g , where g = control or CPSM group;

βt = fixed effect of time t

$(\alpha\beta)gt$ = fixed interaction of effect of group g and time t ;

$\gamma(\alpha)cg$ = random effect of calf c nested within group g ; and

$ecgt$ = error associated with measurements taken from calf c from group g at time t .

Significance will be accepted if $p < 0.05$ and tendencies at $p < 0.10$.

4.6. Results

The average of DMI, BW, DMI/BW and BWG of calves was not significant among treatments in six weeks, 305.4 g/d; 39.4 kg; 7.7 g/d per BW and 32.1 kg, respectively (Table 4.1). The initial BW did not differ among calf groups 22.2 ± 1.49 kg, but the weaning BW was significantly higher ($p < 0.05$) for calves fed CPSM compared to control fed calves and was similar between calves fed Lact and control calves. Calves in Lact and CPSM groups had similar ADG and HG, which were higher ($p < 0.05$) than control calves. Calves in CPSM treatment had higher ($p < 0.05$) HW and SH than control fed calves, but the HW were similar between Lact and other treatments. The HH was higher ($p < 0.05$) in Lact treatment than the rest of treatments, but did not differ between Control and CPSM. There were significant effects of time ($p < 0.001$) for starter DMI (g/d and g/d BW), ADG and all structural body parameters. Furthermore, the interaction effects ($p < 0.05$) between time and treatments for starter DMI (g/d and g/d BW), ADG and HG were observed. The results of calf starter dry matter intake are presented in Figure 4.1.

Table 4.1: Starter feed intake, live weight and structural growth parameters of control calves, calves fed *Lactobacillus acidophilus* (Lact) and *Carica papaya* seed meal (CPSM)

Parameters	Treatments			SEM	Effect of	
	Control	Lact	CPSM		Time	Treatment x Time
Starter DMI, g/d	298.7	320.4	297.1	18.02	<0.001	0.43
Av BW, kg	37.5	40.7	40	1.2	-	-
DMI/BW, g/d/BW	7.9	7.9	7.4	0.4	<0.001	0.04
Initial BW, kg	24.7	21.2	20.7	1.49	-	-
BWG, kg	26.6 ^b	34.00 ^a	35.58 ^a	2	-	-
Weaning BW, kg	51.2 ^b	54.6 ^{ab}	56.7 ^a	2.29	-	-
ADG, kg	0.63 ^b	0.81 ^a	0.85 ^a	2	<0.001	0.034
HG, mm	75.2 ^b	77.7 ^a	77.6 ^a	0.53	<0.001	0.04
HW, mm	76.6 ^b	78.2 ^{ab}	79.7 ^a	0.94	<0.001	0.94
HH, mm	72.4 ^b	75.1 ^a	72.4 ^b	0.56	<0.001	0.06
SH, mm	65.1 ^b	63.9 ^{ab}	66.8 ^a	0.85	<0.001	0.08

SEM: Standard error of mean; ADG: Average daily weight gain; DMI: Dry matter intake; DMI/BW: Dry matter intake per body weight; BW: Body weight; BWG: Body weight gain; HG: Heart girth; HW: Hip width; HH: Hip height; SH: Shoulder height

^{a, b} Means in the same row with different superscripts are significantly different (p<0.05)

All calves increased intake of starter DM (Figure 4.1) from week 1 to week 6. The feed consumed at weaning stage was approximately three times more than the first week. The DMI did not differ among treatments during week 3; 5 and 6 and averaged, 259.70; 427.0 and 495.1 (g/d), respectively. Calves fed Lact consumed more feed than all other calves during week 1, but in week 2 and 4, they had higher (p<0.05) DMI than those fed CPSM, but did not differ with control calves.

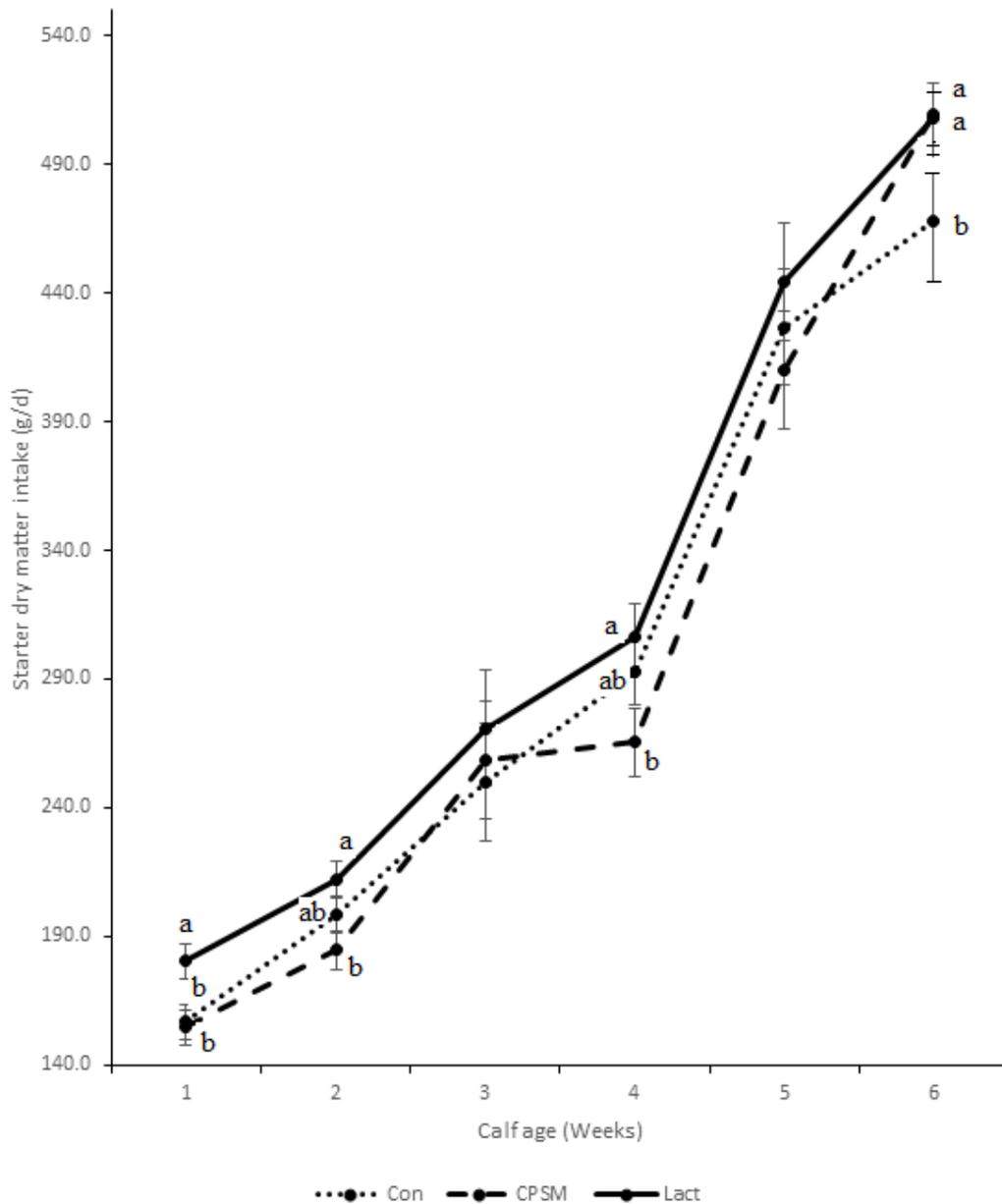


Figure 4.1: Daily dry matter intake of control fed calves, calves fed *Lactobacillus acidophilus* (Lact) and *Carica papaya* seed meal (CPSM) during six weeks.

Calves BW increased from birth to weaning are presented in Figure 4.2. Birth weight of calves did not differ amongst control; Lact and CPSM treatments and averaged 24.7; 20.7 and 21.2 (kg), respectively. The body weight gain of calves did not change among treatments in week 1. In week 3 and 4 calves fed Lact had higher ($p < 0.05$) BW than control calves, but did not differ with CPSM fed calves. No difference in BW was

observed in week 5. In week 6, body weight of Lact fed calves was higher than the control fed calves, but no difference was observed between CPSM fed calves.

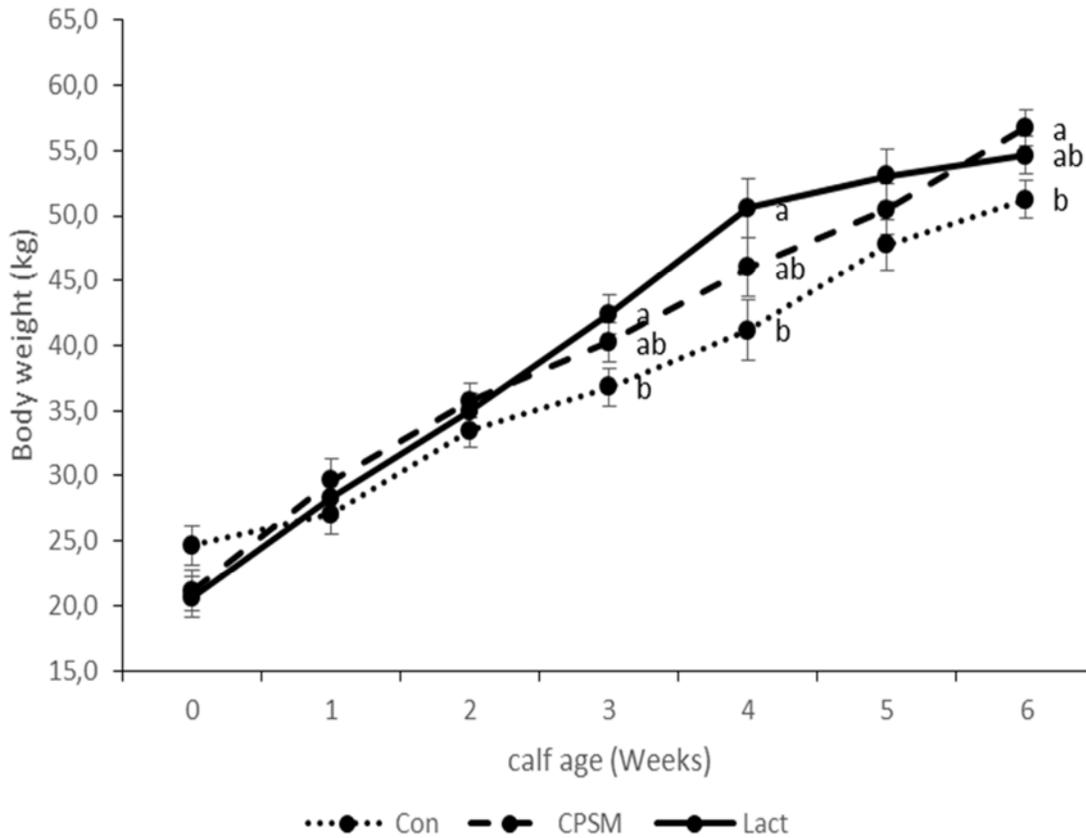


Figure 4.2: Weekly body weight (BW) parameters of control fed calves, calves fed *Lactobacillus acidophilus* (Lact) and *Carica papaya* seed meal (CPSM).

The ADG (Figure 4.3) showed that all calves gained the most weight in week 2 and this was the highest ADG. After week 2 the ADG decreased as the age progressed and reached the lowest value in the last week (week 6) before weaning. During the first week, Lact and CPSM fed calves gained similar weight (0.8 kg/d), which was higher compared to control fed calves (0.70 kg/d). No difference in ADG was found in week 2, where all calves gained an average of 0.90 kg/d. In week 3 calves that received Lact had higher ADG (0.80 kg/d) than the other groups (control and CPSM) where the ADG average was 0.66 kg/d. In week 4, calves fed Lact and CPSM had similar ADG (0.74 kg/d) which was higher than control fed calves (0.63 kg/d). No differences were observed in week 5, but the ADG of Lact and CPSM fed calves

remained numerically higher than control calves. In week 6 the ADG of calves fed CPSM (0.69 kg/d) was higher than the Lact and control groups (0.54 and 0.52 kg/d) respectively.

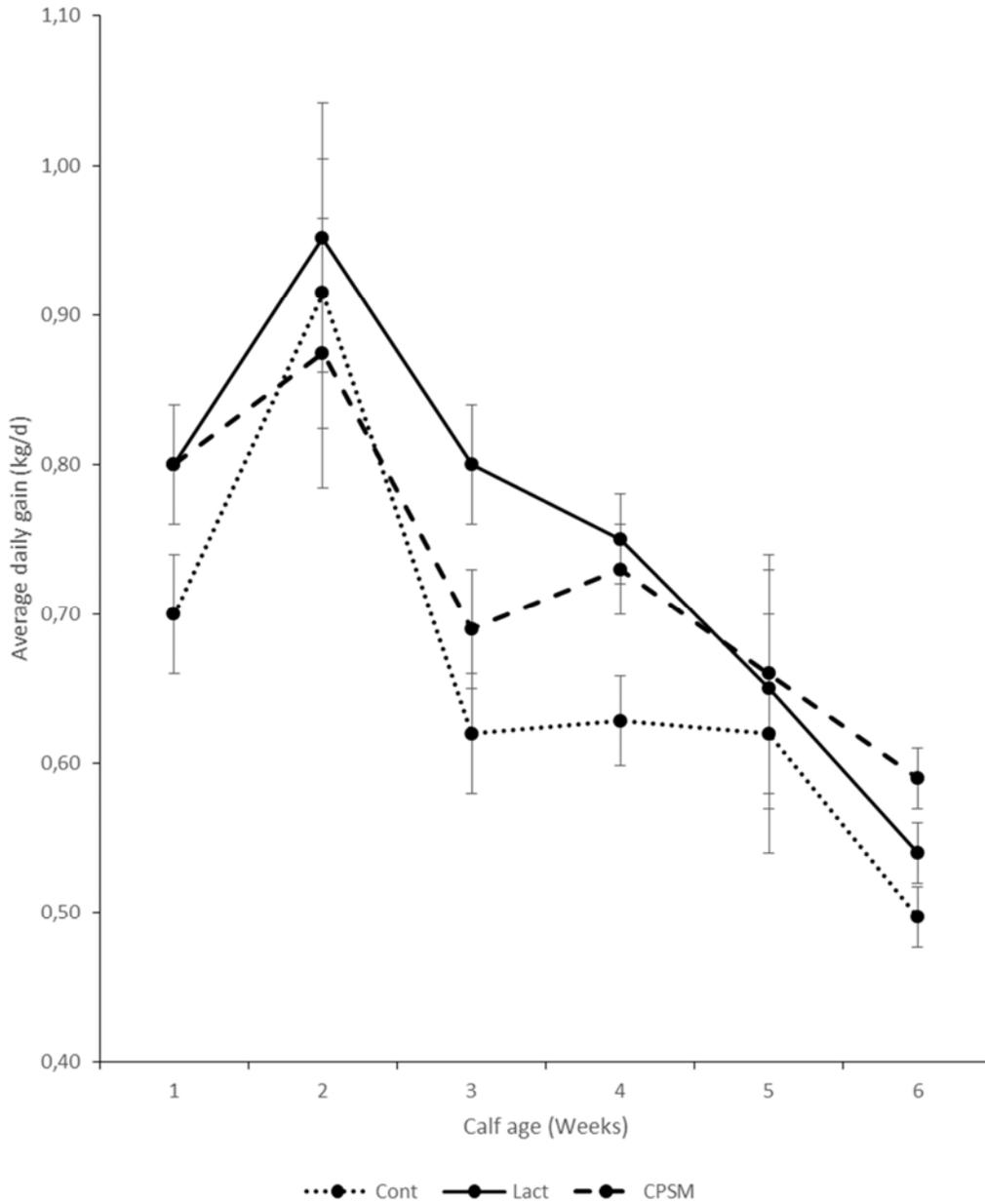


Figure 4.3: Average daily gain (kg/d) of control fed calves, calves fed *Lactobacillus acidophilus* (Lact) and *Carica papaya* seed meal (CPSM).

The results on the change in body structural parameters are presented in the Table 4.2. No differences were observed for all initial (week 0) and final (week 6) structural parameters measured in all groups. From week 1 to 5, the HG of Lact and CPSM fed

calves were higher ($p < 0.05$) than the control. The HW was higher for calves fed CPSM than control fed calves from week 2 to week 4, but did not differ between Lact fed calves, and in week 3 Lact and CPSM fed calves did not differ.

Table 4.2: Weekly measurements of structural growth parameters of control calves, calves fed *Lactobacillus acidophilus* (Lact) and *Carica papaya* seed meal (CPSM).

Parameters	Treatments			SEM
	Control	Lact	CPSM	
Heart girth (cm)				
Week 0	64.58	65.75	66.58	0.95
Week 1	69.41 ^b	72.61 ^a	72.56 ^a	0.77
Week 2	71.88 ^b	76.03 ^a	76.30 ^a	0.71
Week 3	74.30 ^b	78.58 ^a	78.78 ^a	0.99
Week 4	76.60 ^b	81.16 ^a	80.95 ^a	0.61
Week 5	81.18 ^b	83.32 ^a	82.55 ^a	0.57
Week 6	86.66	86.56	85.70	0.68
Hip width (cm)				
Week 0	67.16	67.38	68.16	0.99
Week 1	70.71	71.95	73.12	1.21
Week 2	73.07 ^b	75.70 ^{ab}	78.32 ^a	1.43
Week 3	74.78 ^b	78.98 ^a	81.20 ^a	1.16
Week 4	78.61 ^b	81.18 ^{ab}	83.68 ^a	1.44
Week 5	83.02	83.60	84.15	1.50
Week 6	87.53	88.63	89.70	1.43
Hip height (cm)				
Week 0	67.20	67.32	65.67	0.85
Week 1	68.83	70.55	69.16	0.67
Week 2	70.01 ^b	73.54 ^a	70.55 ^b	0.67
Week 3	71.80 ^b	75.98 ^a	72.13 ^b	0.73
Week 4	73.30 ^b	77.75 ^a	74.68 ^b	0.68
Week 5	76.67 ^b	79.32 ^a	76.75 ^b	0.66
Week 6	78.86	80.85	78.15	0.95
Shoulder height (cm)				
Week 0	54.60	54.48	54.33	1.29
Week 1	57.76 ^b	56.05 ^b	60.88 ^a	0.97
Week 2	63.73 ^a	59.31 ^b	63.96 ^a	1.09
Week 3	65.11 ^b	63.86 ^b	67.96 ^a	0.91
Week 4	67.93 ^{ab}	66.56 ^b	71.48 ^a	1.23
Week 5	71.40	72.50	73.56	1.15
Week 6	74.91	75.12	75.23	1.04

^{a, b} Means in the same row with different superscripts are significantly different ($p < 0.05$)

The HH was higher for calves fed Lact than control and CPSM fed calves from week 2 to week 5, but did not differ between control and CPSM fed calves. The SH was

higher for calves fed CPSM than control and Lact fed calves in week 1 and week 3. In week 2, the SH of control fed calves was similar to calves fed CPSM, averaging 63.85 cm and higher than Lact fed calves (59.31cm). In week 4, the calves fed CPSM had higher SH than calves fed Lact, but no differences was observed between control and Lact fed calves.

4.7 Discussion

Milk intake was not measured, but all calves were left to suckle from their respective dams for the same duration (30 minutes) for each feeding. Therefore, the intake of milk was assumed to be similar for all calves, and differences that may have occurred between calves were considered non-significant. Calves in all three treatments (Con, Lact and CPSM) consumed the same quantity of calf starter. However, all calves fed Lact and CPSM treatments increased BWG and ADG. These calves gained 28.6 (Lact) and 34,9 % (CPSM) more weight weekly resulted in average of 6.0 kg heavier in final weight compared to control fed calves. In the current study, feeding probiotics and CPSM increased feed efficiency and the average daily gain of the calves, although milk and starter consumption was not influenced. Sun *et al.*, 2005; Teo & Tan, 2005 also reported that probiotics increased feed efficiency and average daily gain.

Calves that received CPSM consumed less starter dry feed than all other groups, but the weight gain and final weight of CPSM fed calves were higher than other treatment groups. The CPSM fed calves had an improvement from week 3 to 4 and subsequently remained constant compared to the other treatments which had a decline until the end of the experiment (six weeks). Furthermore, calves improved ADG from week 3 until the end of the experiment (6 week). This might be due to time needed by calves to adapt to the additives before showing the effects. A week before (week 5) weaning calves fed CPSM gained 0.69 kg/d while Lact and control groups gained 0.54 and 0.52 kg/d respectively. Study by Farrag *et al.*, (2013) reported that papaya seed meal at different levels significantly increased growth performance and feed utilization. In the current study, the ADG of control and Lact calves decreased around weaning, as reported in other studies (Jasper & Weary, 2002; Terré *et al.*, 2006). The decrease of ADG seven days before weaning is believed to be associated with the decrease in energy and protein intake when milk quantity was reduced before weaning. However, CPSM calves maintained their ADG, which is of great benefit in early weaning system

of calves. This might be attributed with the nutritional composition present in the papaya seeds. This is confirmed by Marfo *et al.*, (1986) & Krishna *et al.*, (2008) who observed that papaya seeds are rich sources of nutrients. Early weaning is always recommended in the cattle industry to save costs and increase profit, but more often resulted in depressed growth performance after weaning (Sun *et al.*, 2010).

Control and Lact fed calves consumed more starter feed than CPSM fed calves, but their weight gained and final weight were lower. Previous studies emphasised that the importance of probiotic supplements to young and stressed calves is to establish and maintain intestinal microbial balance rather than production (i.e., gain, feed intake and efficiency) stimulants (Krehbiel *et al.*, 2003; Malik & Bandla, 2010). Timmerman *et al.*, (2005) and Cruywagen *et al.*, (1996) observed that administration of *L. acidophilus* positively affected growth performance after birth. In the current study, the growth-promoting effects of probiotics did not persist for long while treatment was continued. It is suggested that possibly after administering the probiotics, calves were adapted to change in diet and other environmental factors. Therefore, probiotic treatments become less effective after a certain period of administration. The positive effects of probiotics on growth performance of calves may only be present when their health status has improved. The results of supplementing with probiotic on body weight and growth have been controversial. The results by Bernardeau *et al.*, (2006) indicated that animals treated with *L. acidophilus* strains shown significant increased BWG. However, others shown to be species dependent and some species had negligible effects on body weight or reduced it (Million *et al.*, 2012).

This net improvement of calf ADG with feeding CPSM is similar to the observation of Farrag *et al.*, (2013). The feeding of papaya seed powder for 45 days had positive results on ADG compared to control. In addition, the rate of growth expressed as the percentage increase in body size was the highest in the papaya seed fed calves. According to Osato *et al.*, (1993) and Farrag *et al.*, (2013) the improvement in growth performance caused by papaya seed powder is related to its bacteriostatic effects against several gram positive and gram negative organisms. Furthermore, *Carica papaya* seeds contain numbers of bioactive compounds that have anthelmintic (Kermanshai *et al.*, 2001), and antiparasitic activities (Hounzangbe-Adote *et al.*, 2005) that prevent the establishment of opportunistic pathogenic bacterial populations in the

gut. Another consideration is that the growth can also be attributed to improved nutrient utilisation due to increased nutrient digestibility and improved ruminal fermentation activities (Muya *et al.*, 2015). Krishna *et al.* (2008) indicated that papain in papaya seeds increased protein digestibility, which is positively related to calf growth rate, because maintenance requirements for protein are negligible. Although the ADG of all calves decreased from week 2, the decrease was more pronounced in Lact and control fed calves. From week 2 to 3, in the control group, ADG decreased by approximately the double the ADG of the rest of the treatments. The CPSM and Lact fed calves gained 8 % more BW per kg of feed consumed than control calves.

The structural body growth of all treatments calves did not differ in the first week after birth, but significantly changed from week 2 to 5, in Lact and CPSM fed calves. This confirmed the better body growth and body condition score. The structural body growth of all calves during the last week before weaning declined steadily in the present study, and it is in agreement with Kertz *et al.*, (1998).

A study by Lee *et al.* (2000) indicated that gastrointestinal tract of young ruminants at birth is physiologically immature compared with that of adult ruminants. The growth is stimulated when young calves consumed starter feed and water at early age. Quigley *et al.* (2001) indicated that provision of sufficient water and dry feed, particularly starter influenced the ruminal development and growth of bacteria. This influenced by butyrate production known as the most stimulatory of the VFAs produced in regards to development of papillae (Tamate *et al.*, 1962; Muya *et al.*, 2015).

4.8 Conclusion

The present study demonstrated that *Carica papaya* seed (*Linn*) meal and *L. acidophilus* gave similar results on increased animal performance by improving average daily gain and feed efficiency of calves during the pre-weaning period. Further studies are needed to ascertain the effects of additive graded levels on animal performance and feed utilization of calves.

CHAPTER 5

GENERAL CONCLUSION

In the current project, two studies were performed to investigate the effects CPSM on faecal pathogens and growth performance of dairy calves.

Most problems that affect growth performance of calves are associated to poor digestion and compromised nutrient absorption because pathogenic colonization of bacteria. Supplementing with CPSM can be considered as a supplement to prevent

the prevalence of diseases such as diarrhoea, which are mainly caused by pathogens colonized on the digestive tract. The effects of *L. acidophilus* on stabilizing rumen bacterial population and preventing colonisation of pathogens can be considered as the main cause of growth improvement observed in the current study. The *L. acidophilus* will continue to play a role in calves. *Carica papaya* seed meal can also be considered for reducing the load of pathogens of the digestive tract, and reduce the prevalence of infectious diseases. Additionally, *Carica papaya* seed meal may be an alternative to the use of antibiotics as a growth promoter in calves.

The first study showed that feeding *Carica papaya* seed meal on dairy calves early after birth reduced the faecal count of coliforms, *E. coli* and *Enterobacteriaceae*, and increased total dry matter and nutrient intake. These results suggested that papaya seeds possess anthelmintic and antimicrobial properties against the gut pathogens as observed in several other studies. Consequently, a better utilisation of feed nutrients from both calf starter feed and milk was observed. The second study showed that the average daily gain of weight was improved. The CPSM resulted to heavier calves at weaning age (42 days). Feeding *L. acidophilus* also improved growth but showed lower feed efficiency than CPSM. These results showed the benefits of CPSM on young calves, as they are more susceptible to infectious diseases, by minimizing pathogenic bacteria colonization of the digestive tract.

However, further research is warranted to document effects of feeding CPSM on calves when feeding measured milk, effects on blood parameters and long-term effects.

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