Effect of a natural fermentation liquid supplement on health, performance, carcass characteristics and rumen fermentation dynamics in beef feedlot cattle

by

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Submitted in partial fulfillment of the requirements for the degree

M.Sc. (Agric.): Animal Science: Animal Nutrition

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Declaration

I, Jonathan Clive Miles, declare that this dissertation/thesis for the degree M.Sc. (Agric.) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Summary

Effect of a natural fermentation liquid supplement on health, performance, carcass characteristics and rumen fermentation dynamics in beef feedlot cattle

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As feed costs continue to rise, new technologies that will improve feed efficiency in a safe and consistent manner will become more critical for beef cattle production. With this in mind, two experiments were conducted. Experiment 1, a randomized complete block design study utilizing 144 Bonsmara-type steers (233 ± 0.8 kg BW) was conducted to examine the effect of a liquid *Lactobacillus* fermentation prototype (LFP; Diamond V, Cedar Rapids, IA) on performance of feedlot cattle. The 134 day study was conducted on a commercial beef research facility in South Africa. Upon arrival, cattle were processed and allocated to a free-range pasture for 8 d. On d 9, cattle were further processed and then blocked by arrival body weight and randomly assigned to one of 3 treatments with 8 pens per treatment and 6 steers per pen. Dietary treatments included a control diet that contained 0, 5, or 10 g LFP per head daily. Treatments were provided a in starter (d 9 to 29), grower (d 30 to 43) and finisher diet (d 44 to 134). The cattle were then slaughtered and carcasses data obtained. Experiment 2, a 3x3 Latin Square design conducted with three Beefmaster steers (700kg ±20kg) fitted with ruminal cannulae to establish a possible mode of action of the LFP supplement. Rumen fermentation parameters namely VFA concentration, rumen NH_3-N, ruminal pH, lactic acid concentration and NDF disappearance were measured. In experiment 1 no differences were detected ($P > 0.05$) among treatments for BW gain, ADG, DMI or any of the carcass traits that were measured. Overall, (d 9 to 134), FCR was improved ($P = 0.03$) for 5 g LFP supplemented steers compared with those receiving 0 or 10 g LFP (4.70 vs. 4.82, 4.86). No differences in FCR, however, were reported, between treatment groups, in each individual feeding phase. Results from experiment 1 suggest that LFP does improve feed efficiency of beef cattle fed a typical South African feedlot diet and that the effect was dose dependent. Results from experiment 2 showed no differences in any of the rumen fermentation parameters that we measured. Further research on the mode of action of LFP is warranted.

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List of Abbreviations

ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
CF	Crude fibre
CP	Crude protein
Da	Dalton
DFM	Direct-fed microbials
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
FCR	Feed conversion ratio
GE	Gross energy
IVOMD	*In vitro* organic matter digestibility
LFP	Liquid fermentation prototype
ME	Metabolizable energy
MJ	Mega Joule
NDF	Neutral detergent fibre
NFC	Non fiber carbohydrate
NSC	Non-structural carbohydrate
SEM	Standard error of the mean
TMR	Total mixed ration
VFA	Volatile fatty acid

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Chapter 1

Introduction

Cattle feedlots have over the years been faced with numerous challenges impacting on potential feedlot profitability. These included negative effects of stressed newly received cattle, morbidity, mortality, fluctuations in major ingredient prices, oscillation in beef price, variations in ingredient quality and availability just to name a few. As these challenges became more imminent feedlot operators were continuously striving for improved efficiency, particularly feed efficiency. Since the approval of ionophores and antibiotic growth promoters (AGP) in the mid-1970's feed efficiency in beef cattle has improved dramatically. Several ionophores and AGPs have since been discovered and approved as feed additives for beef cattle. Due to the cost effectiveness and efficacy ionphore adoption in feedlots increased rapidly to the point that almost all feedlots in South Africa include an ionophore and/or an AGP in their diets (D. Ford, *personal communication,* South African feedlot association, 2011). Obvious exceptions are those targeting "natural" or "organic" beef production.

Recently, however, negative public perception over the use of antimicrobials in animal agriculture has dramatically increased, initiating in the European Union (EU) and United States of America (USA). This has lead to research efforts being concentrated on the development of alternative feed additives such as direct-fed microbials (DFM), essential oils, enzymes and organic acids. The target of development and research was to achieve similar responses in production parameters to that commonly associated to ionophore and AGP supplementation. January 1st 2006 marked the EU-wide ban for AGP and ionophore supplementation (Official Journal of European Union), since then consumers worldwide are placing more and more pressure on feedlot operators to phase out ionophores and AGP's. Unfortunately for feedlot operators this normally comes at a grave cost implication as most of the alternative feed additives are substantially more expensive that ionophores and AGPs. In addition to higher input cost of the more expensive additives, profitability has decreased due to the fact that the majority of the alternative feed additives achieve inferior production responses to the ionophores and AGP's commonly used (D. Ford, *personal communication,* South African feedlot association. 2011). It is also frequently reported in literature that over and above the improvement in production parameters ionophores and AGPs offer, they are also very effective against metabolic disturbances such as bloat, acidosis and liver abscesses (Patra, 2011). These responses are not shared by the group of alternative feed additives. Although ionphores and AGPs are still, to date, permissible for use in South Africa; public perception and consumer driven demands are stimulating feedlot owners and nutritionists to explore alternative feed additives.

Recent research has mainly focused on exploiting the use of plant metabolites and/or DFM's to manipulate rumen fermentation. Ryan and Gray (1989) and Wallace and Newbold (1995) showed that yeast DFMs could be used as rumen modifiers, possibly altering volatile fatty acid (VFA) production as well as enhancing protein metabolism and uptake. *In vitro* studies done by Cardozo *et al.* (2004) and Busquet *et al.* (2005, 2006) confirmed that plant metabolites can be beneficial as rumen modifiers. Beauchemin *et al.* (2003b) and Krehbiel *et al.* (2003) showed that bacterial DFM's could both manipulate rumen fermentation patterns as well as alleviate possible metabolic disturbances such as acidosis. Despite large inconsistencies in results being obtained from *in vivo* experiments done on alternative feed additives, positive effects appear to support their proposed mode of action and warrant investigation (Haasbroek, 2013).

Therefore the objectives of this study were: (i) to determine the effect of an all-natural liquid fermentation prototype on the performance, health, and carcass characteristics of feedlot cattle and (ii) to determine the possible mode of action by accessing rumen fermentation parameters.

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An overview of the role of rumen modifiers will be presented in the next chapter followed by two chapters where firstly the effect of a liquid fermentation supplement on growth and health was investigated and secondly the effect on rumen fermentation dynamics.

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Chapter 2

Literature Review – The role of ionophores, antibiotic growth promoters and direct fed microbials in ruminant diets

1. Introduction

The intensive feeding of cattle, commonly referred to as feedlotting, is common practice throughout the world. Despite differences in feeding practices, diets fed, feeding duration and end product (carcass) grading systems, the common goal across all countries is to maximize growth efficiency and to minimize the cost of carcass gain. This can only be achieved by the feeding of high concentrate diets. Due to their natural rangeland habitat; ruminants have evolved and are aptly suited to effectively utilize very fibrous feedstuffs (Sudweeks *et al.,* 1981). The supply of energy from more nutrient dense sources, such as grains, can be considered detrimental for the reticulo-rumen environment. Therefore ruminants are prone to develop digestive disorders when fed high concentrate or grain diets (Krause and Combs, 2003). Digestive disorders, such as acidosis, bloat and liver abscesses are frequently observed in feedlots (H. J. Vermaak, *personal communication,* Essential Nutrient Systems, 2012). Feed additives have been used in both South Africa as in other countries to firstly improve feed efficiency and secondly to reduce the incidence of these digestive disorders.

In this chapter an overview of the most commonly used feed additives in the South African feedlot industry will be given with emphasis on mode of action, effects on ruminal N and energy utilization and the effects on animal performance and health.

2. Different types of rumen modifiers

2.1 Ionophores

Ionophores are organic compounds largely from the *Streptomyces* species that assist in the interruption of transmembrane movement and intracellular equilibrium of ions, mainly potassium, hydrogen and sodium, in certain classes of bacteria and protozoa that inhabit the gastrointestinal tract (McGuffey *et al.,* 2001). This gives a competitive advantage for certain microorganisms at the expense of others (Hobson and Stewart, 1997).

Gram-positive bacteria lack the complex cell wall of Gram negative bacteria and its associated lipopolysaccharide layer that contains porins, which are protein channels that have an exclusion limit of 600Da. Ionophores size is greater than 600Da therefore impervious to ionophores (McGuffey *et al.,* 2001). Consequently, ionophores successfully infiltrate the outer cell membrane of Gram-positive bacteria and quickly implicate their mode of action, refer to 1.1.1a) All ionophores share a common mode of action, but some differences, e.g. cation specificity and potency (capacity to achieve rumen concentrations, exist among the molecules (Pressman, 1976; Chow *et al*., 1994). Differences in the relative potency of the various ionophores on bacterial and protozoal cell metabolism can be at least partially explained by the complexation of cations (Westley, 1978), refer to 2.1.1 a) and 2.1.2.a). Most are approved in the prevention of coccidiosis, however they are mainly added to ruminant diets to improve feed efficiency and/or increase performance.

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2.1.1 Monovalent ionophores

a) Mode of action

Monovalent ionophores attach to bacteria and protozoa (Fitzgerald and Mansfield, 1973; Chow *et al*., 1994) and according to (Habib and Leng, 1987) most probably rumen fungi as well. The ionophores imbed in the lipid bilayer where it facilitates the movement of cations across the lipid bilayer of the cell. Therefore, exchanging one monovalent cation for one proton, referred to as a metal/proton antiporter (Pressman, 1976) these reactions occur very rapidly and disrupt the cation-anion balance between intracellular and extracellular regions of the cell. As referred above the relative potency of ionophores can partially be explained by the complexation of cations. Monovalent ionophores have a relatively small chelation cavity; monensin for example has a ten-fold comparative affinity for Na^+ over K^+ (Guffanti *et al.,* 1979; McGuffey *et al.,* 2001) and no affinity for large ions such as Ca^{2+}. This is extremely advantageous as the transportation of ion(s) e.g. Na^+ and/or H^+ in one direction (into the cell) and the other K^+ out of the cell, this other leads to a disruption extracellular-intracellular ionic gradient. Protons accumulate inside the bacterial cell. (Pressman, 1968; Schwingel *et al*., 1989). The efflux of intracellular K^+ ions from the cell and an influx of extracellular protons (Na^+ and H^+) cause acidity and to overcome this, and the depletion of K^+ ions, which inhibits protein synthesis, proton ATPase and sodium ATPase activity increases. (Guffanti *et al.,* 1979). The depletion of ATP results in inhibition of cell division and bacterial growth. Depletion of ATP also results in the inability for the bacteria to alleviate the acidic conditions in the cause cell death. However, at common commercial levels in ruminant diets, ionophore mediated reductions in ATP and in K+ renders the organism incapable of sustaining a rate of cell division sufficient to maintain normal metabolic significance (Russell and Strobel, 1989).

b) Effects on ruminal N and energy utilization

As described in United States of America patent filing, Raun (1974) discovered that sodium monensin, a common monovalent ionophore, enhances rumen propionate production, at the expense of acetate production, and was able to implement this biological action into the first commercial application of ionophores for the cattle industry in 1975. Much of the focus of ionophore research in the 1970s and 1980s concentrated on changes in VFA production when ionophores were fed because of the emphasis on energy utilization in beef feedlot diets. Due to the fact that Gram-positive bacteria mainly ferment dietary nutrients in to products such as acetate, methane and carbon dioxide Table 7.1, monovalent ionophore treatment improves the rumen function and animal performance by reducing the production of these metabolites. Sodium monensin was shown, measured by isotope dilution, in four experiments to increase propionate production rates by 49 % in steers fed forage and 76% when being fed concentrate rations (Van Maanen *et al.,* 1978). Similarly Rogers and Davis (1982), showed monensin fed with a maize silage and concentrate ration increased propionate production by 65% and with a hay and concentrate ration by 44% (Prange *et al*., 1978). As depicted in Table 7.2 Dinius *et al.* (1976) showed an increase of 30% in propionate production. The increase in propionate production is at the expense of acetate, thus, and as can be seen in Table 7.2, monovalent ionophores reduce the acetate to propionate ratio and hence improves the utilization of energy in the rumen. The increases in the molar proportion of propionate in the first three mentioned experiments were smaller than the changes in production rates, indicating that measurements of VFA concentration in rumen fluid can underestimate the effects of monovalent ionophores on VFA production (McGuffy *et al.,* 2001.). Enhanced energy efficiency of fermentation caused by the addition of sodium monensin is demonstrated very well by the work of Rogers and Davis (1982). Beef steers were fed a diet of 50% maize silage and 50% concentrate, control versus treatment with sodium monensin (33 mg/kg DM).

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The treatment group showed that sodium monensin increased the daily ruminal production of acetic, propionic, and total VFA's per kg DM intake by 29, 64, and 35%, respectively. The total energy produced in the form of VFA's in the rumen per kg of DM consumed was increased from 3.564 MJ/kg of DM for control steers to 4.757 MJ/kg of DM for steers fed the treatment diet, an increase of 33% in ruminal digestible energy (Rogers and Davis 1982).

Russel and Strobel (1989) reported that with the treatment of monovalent ionophores, the addition of monensin, ruminal methane production can be reduced by up to 30% by inhibiting bacterial species providing the precursors of methane (formate and H_2) rather than directly inhibiting methanogenic bacteria (Dellinger and Ferry, 1984.). Bacteria that produce these precursors are fairly sensitive to monovalent ionophores, whereas methanogens are more resistant (Chen and Wolin, 1979) and represented in Table 7.1. This is particularly important as McGuffy *et al.* (2001) reports that up to 12% of gross energy of feeds can be lost as eructated methane. A shift in hydrogen metabolism to more "valuable" end products not only captures more digestible energy from fermented organic matter, thus more efficient, but also reduces methane emission into the atmosphere. According to recent research done by Odongo *et al.* (2007), the use of sodium monensin reduced methane emissions by almost 10 percent in dairy cows. Research by Tedeschi *et al.* in 2003 suggests monensin may mitigate methane emissions in beef cattle by up to 25 percent.

As early as the 1970s the effect of ionophores on nitrogen metabolism was recognised, mainly by research done by the likes of Dinuis *et al.* (1976) and Van Nevel and Demeyer (1977). Much of the research concluded that monovalent ionophores, reduced *in vitro* protein degradation, ammonia accretion and microbial nitrogen concentration (Van Nevel and Demeyer, 1977.). However, upon the identification of a *Peptostreptococcus* species and a *Clostridium* species by Russell *et al.* (1988) it was shown that the above mentioned observations were not due to reduced proteolysis but rather due to the inhibition of *Peptostreptococcus* species and *Clostridium* species by monovalent ionophores (in this case sodium monensin) both Gram positive bacteria and both with a very high rate of ammonia production in the rumen. The reduction of ammonia production results in an accumulation of nonammonia, nonprotein nitrogen in the form of amino acids and short peptide chains. Both *Peptostreptococcus* species and *Clostridium* species require amino acids and peptide chains as an energy source for growth. Thus a subsequent increase in the amount of dietary nitrogen reaching the abomasum was shown (Faulkner *et al*., 1985). The observed reduction in microbial nitrogen is due to the significant decrease in ruminal bacterial protein synthesis. The efficiency of thereof was however unchanged (Faulkner *et al*., 1985.).

c) Effects on animal performance and health

Most monovalent ionophores are very effective at preventing the incidence of coccidiosis in ruminants. As indicated previously in this chapter monovalent ionophores inhibit the growth of lactate-producing bacteria, (Dennis *et al.,* 1981) such as *S. bovis* and *Lactobacillus* spp, while most lactate utilizing bacteria remained unaffected. These changes in the rumen microbial population and subsequent shift in ruminal fermentation were shown to be effective in alleviating ruminal pH in both beef cattle (Nagaraja *et al*., 1985; Burrin and Britton, 1986; Cooper and Klopfenstein, 1996) and dairy cattle (Green *et al*., 1999) fed diets high in grain. Significant increases in ruminal pH would result in favourable conditions for cellulolytic bacteria growth, which could increase fiber digestibility. There is, however, limited supporting literature in which ruminal degradability and total tract digestibility of fiber measurements under subclinical acidosis conditions (Osborne *et al.,* 2004).

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Duffield *et al.* (2008) reported that due to the increased glucose precursor supply mainly in the form of elevated production of ruminal propionate and amino acids, sodium monensin can reduce the risk of ketosis and fatty liver syndrome in lactating dairy cows.

" Grain" bloat as a result of foam build up in the reticulum and rumen, and failure to eructate, is a major concern in not only South African feedlots but in other parts of the world. Acute abdominal distension as a result of the bloat often leads to a rapid death if not treated (McGuffey *et al.,* 2001). Sakauchi and Hoshino (1981), showed that the viscosity of rumen fluid from steers predisposed to bloat was higher than in normal steers fed a feedlot type diet. The observation of bloat was 86.3% during a 30 day feeding period. Supplementation of monovalent ionophores, in this case sodium monensin, reduced the incidence of bloat to 4.2% over the following 36 days. Thereafter sodium monensin supplementation ceased and the incidence increased to 24.3% in the next 36 days. During the supplementation phase of sodium monensin the viscosity of rumen fluid of the steers decreased to values similar to that observed in normal steers unaffected steers (Sakauchi and Hoshino, 1981). Usagawa (1992) observed similar effects of sodium monensin on viscosity of rumen fluid in sheep.

2.1.2 Divalent ionophores

a) Mode of action

Divalent ionophores, of which lasalocid is the most commonly known, have a similar mode of action to that of monovalent ionophores described previously in this chapter. This being that divalent ionophores act primarily on Gram-positive bacteria they have limited antimicrobial capacity on Gram-negative bacteria. This is due to their size of greater than 600Da therefore not being able to pass through the porins of the Gram-negative cell wall (McGuffey *et al.,* 2001). The difference in mode of action between monovalent and divalent ionophores is due to the ionophores affinity and binding capacity to certain cations (Westley, 1978). Monensin has a very high affinity for Na^+ ions whereas lasalocid has a higher affinity for K^+ ions. Lasalocid has also been shown to have significant affinity for divalent cations (mainly Ca^{2+}) and hence the classification as divalent ionophores (McGuffey *et al.,* 2001). Monensin has however a very low affinity for divalent cations hence its classification as a monovalent ionophore. It is important to note that divalent ionophores do not exclusively have to have an affinity to divalent cations as the classification suggests. It is this affinity for certain cations, or lack thereof for others, that results in the significant differences in efficiency of the ionophores on rumen microbes (Pressman, 1968; Pressman 1976; Westley, 1978; Schwingel *et al.,* 1989). Similar changes in ionic gradient and ATPase activity are elicited and therefore the inhibition of Gram-positive bacteria occurs.

b) Effects on ruminal N and energy utilization

Due to a very similar mode of action, on Gram-positive bacteria, between monovalent and divalent ionophores, ruminal fermentation responses are very similar. Volatile fatty acid production and molar proportions yield similar results compared to monovalent ionophores. Divalent ionophores show a shift in acetate to propionate ratio, towards more acetate (Bergen and Bates, 1984). This was in agreement with earlier work done by Mowat *et al.,* in 1977. Authors also reported some increase of lactate metabolism to propionate. Ricke *et al.* (1984) and Fuller and Johnson (1981) showed a significantly greater reduction in butyrate and iso-valerate proportions for lasalocid over monensin. Decreases in methane production due to the lowered availability of the precursors needed for methanogensis (Perksi *et al.,* 1982) rather than inhibiting the growth of methanogenic bacteria. Lasalocid, as with monensin, also shows reduced NH_3-N production, increased proportion of absorbed

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N retained and decreased urinary N (Yang *et al.,* 2003). It was reported that the increase in ADG in the latter experiment was due, in part, to the improved nitrogen utilization.

c) Effects on animal performance and health

Animal performance improvements are very similar to that of monovalent ionphores. In most studies lasalocid did or did not improve ADG, had very little effect on DMI, but showed an improvement on feed efficiency (Owens, 1980; Berger *et al.,* 1981; Mader *et al.,* 1985; Zobell, 1987). Despite performances being very similar, differences have been reported in literature and indicate that ionophore-diet interactions will elicit different responses for different diets. For example differences in fermentation of roughage sources are apparent between mono- and divalent ionophores; the mode of action however is not fully understood. Bretschneider *et al.* (2007) showed that as the quality of forage increased, the net ADG response to monensin decreased and that to lasalocid increased.

As with monovalent ionophores divalent ionophores have been shown to inhibit the growth of major ruminal lactate producers (Dennis *et al.,* 1981a,b). Therefore lasalocid reduces the risk of rapid ruminal pH drop when cattle are fed high concentrate diets. Nagaraja *et al.* (1981, 1982) showed that both monensin and lasalocid are effective in preventing lactic acidosis if steers were induced with acidosis. Nagaraja *et al.* (1981, 1982) also reported that lasalocid appeared to be more effective against lactic acid production than monensin. The authors suggested that this was due to a superior inhibition of *S. bovis* of the lasalocid over the monensin.

Lasalocid has been shown to be effective against coccidiosis, therefore reducing the chance of *Eimeria spp.* during periods of high stress such as receiving and processing (Sakauchi and Hoshino, 1981). Lasalocid has also been shown to decrease rumen viscosity in bloated animals (Bergen and Bates, 1984).

2.2 Antibiotic growth promoters

2.2.1 Virginiamycin

Virginiamycin initially entered into the feed industry as a performance enhancer or antibiotic growth promoter (AGP) for swine and poultry. During the late 70's Parigi-Bini (1979) and Hedde *et al.,* (1980) reported beneficial effect of virginiamycin on growth and feed efficiency in cattle. Virginiamycin is a fermentation product of *Streptomyces virginiae*, it is referred to as a peptolide antibiotic and consists of two components namely factor M and factor S (Gottschall *et al.,* 1988).

a) Mode of action

Virginiamycin acts primarily on Gram-positive bacteria, where it penetrates through the cell wall and into the lumen of the bacterial cell. In the cell virginiamycin binds to the 50S ribosomal sub-unit of the bacterial cell, thereby inhibiting protein synthesis through the prevention of the formation of the peptide bond (Cocito, 1979). The two components of virginiamcyin, M and S factors, act synergistically factor S potentiates the activity of factor M (Rogers *et al.,* 1995). The binding of the two components to the ribosomal subunit disrupts the metabolic processes, causing a bactericidal effect. Amongst numerous authors Nagaraja and Taylor (1987) and Nagaraja *et al. (*1987) reported the inhibition of growth of ruminal lactic acid producing bacteria *in vitro*. The inhibition of lactic acid production is commonly associated with alleviation in rumen pH drop in cattle fed a high concentrate diet. This was recently confirmed *in vivo* by Coe *et al.* (1999), the authors reported lower

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Lactobacillus and *S. bovis* counts compared to the control and monensin and tylosin fed groups. Naturally this will lead to the reduced incidence of ruminal acidosis, liver abscesses and depressed feed intake commonly associated with general feedlot diets (Rogers *et al.,* 1995).

b) Effects on ruminal N and energy utilization

Very few studies have been designed to determine the ruminal responses to potential mode of action of virginiamycin. The effect on ruminal microorganisms and fermentation patterns appear to be similar to that of ionophores. These changes included increased molar propionate proportions, decreased lactic acid production, decreased methane production and reduced protein degradation (Nagaraja *et al.,* 1997). Reports from clinical studies, however, seem to be slightly inconsistent. No improvements on ruminal propionate concentrations were reported (Hedde *et al.,* 1980) when cattle being fed an 82% concentrate diet with or without virginiamycin. *In vitro* studies done by Van Nevel *et al.* (1984) later showed increased molar propionate proportions, decreased protein degradation and decreased methane production. Rogers *et al.* (1995) suggests that additional research on the post-ruminal effects of virginiamycin may improve our understanding of the improved growth and efficiency achieved in ruminants supplemented virginiamycin.

c) Effects on animal performance and health

A meta analysis published by Rogers *et al.* (1995) which included data from seven clinical field experiments and using three thousand one hundred beef feedlot cattle demonstrated the animal performance benefits of supplementing Virginiamycin at various levels in beef feedlots. The authors reported improvements on ADG, and feed efficiency with no significant effect on dry matter intake. The improvement of feed efficiency was also reported to be related to the dietary energy concentration. With improvements greatest on diets containing higher metabolizable energy, improvements in feed efficiency can be expected between 2% and 8.4% (Rogers *et al.,* 1995). In addition to dietary energy factors such as the type of grain, the processing method of the grain also seem to influence the response of cattle to virginiamycin (Rogers *et al.,* 1995). It was concluded by Rogers *et al.* (1995) that a consistent response to virginiamycin is an improvement in feed efficiency primarily due to an increase in body weight gain and little or no effect on dry matter intake.

As discussed previously in this chapter, virginiamycin has been shown to reduce the concentration of ruminal lactate, by inhibiting lactic acid producing bacteria (Rogers *et al.,* 1995). Therefore, showing a tendency to reduce the risk of acidosis in the rumen, confirming the observation of a higher ruminal pH made by Hedde *et al.* (1980) in virginiamycin fed steers compared to the control.

Despite the precise mechanism of formation of liver abscesses being relatively unknown, it is well known that acidosis derived rumen lesions are the predisposing factors for liver abscesses (Nagaraja and Chengappa, 1998). *Fusobacterium necrophorum* was reported in the same study to be the primary etiologic cause for liver abscesses. Coe *et al.* (1999) showed a decrease in *F. necrophorum* counts in virginiamycin fed steers compared to the control. This is likely to be as a result of a decreased availability of lactate, a major energy source for *F. necrophorum* (Nagaraja and Chengappa, 1998). Numerous studies (Hedde, 1984; Tan *et al.,* 1994a; Rogers *et al.,* 1995; Nagaraja and Chengappa, 1998) showed a reduction in the incidence of liver abscesses when virginiamycin was fed to beef cattle.

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2.2.2 Tylosin

Incidences of liver abscesses in feedlot cattle range between 12-32% (Brink *et al.,* 1990). It is generally accepted that acidosis induced rumenitis is the predisposing factor for the onset of liver abscesses (Smith, 1944; Jensen *et al.,* 1954a, b; Tan *et al.,* 1996). *Fusobacterium necrophorum* is reported to be the primary etiologic cause for liver abscesses, the control thereof and subsequently the control of liver abscesses is generally dependent on antimicrobials amongst others tylosin (Nagaraja *et al.,* 1996a, b; Nagaraja and Chengappa, 1998). Tylosin is of the macrolide group of antimicrobials.

a) Mode of action

The mode of action of tylosin is reported to be primarily against Gram-positive bacteria, there are however some Gram-negative bacteria that are sensitive to it, including *F. necrophorum* (Berg and Scanlan, 1982; Lechtenberg and Nagaraja, 1989; Tan *et al*., 1994b). The mechanism is similar to that of virginiamycin, as discussed in section 2.2.1. Tylosin binds reversibly to the 50S sub-unit of the bacterial ribosome, inhibiting protein synthesis, causing a bactericidal effect. The antimicrobial effect of tylosin on *F. necrophorum* is thought to primarily be in the rumen (Nagaraja *et al.,* 1996c); it, however, can partially be absorbed through the gut wall (Gingerich *et al.,* 1977) and have an effect on *F. necrophorum* in the liver.

b) Effects on ruminal N and energy utilization

Due to the inclusion of tylosin being mainly for the control of liver abscesses not much research has been carried out on the effect on rumen parameters. Understanding the mode of action of tylosin, a macrolide, on Gram-positive bacteria would suggest that rumen fermentation and degradation parameters should be affected by tylosin. Since both virginiamycin and tylosin act on the bacterial ribosome 50S subunit, referral is made to the effect on ruminal and energy utilization of virginiamycin.

c) Effects on animal performance and health

A meta-analysis of 40 trials involving a total of 6,971 beef feedlot cattle from all major cattle feeding areas of the United States demonstrated that tylosin feeding reduced the incidence of liver abscesses by 73% (Vogel and Laudert, 1994). Cattle fed tylosin have been reported to increase ADG by 2.1%, and an improvement in FCR of 2.6%. The cattle in the studies also tended to yield a higher dressing percentage than the control fed cattle (Vogel and Laudert, 1994).

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2.3 Direct fed microbials

2.3.1 Yeast DFM

Yeasts are single celled fungi that mainly contribute to carbohydrate fermenting in the rumen. Most commonly strains of *Saccharomyces cerevisae* are included in commercial products. Most commercial products contain a combination of various strains live and dead of *S. cerevisae* cells. Those that contain predominantly live *S. cerevisae* are sold as "live yeasts" compared to "yeast cultures" which contain mostly dead cells and the growth medium.

a) Mode of action

The mode of action of yeast cultures and live yeast is multifactorial. Recent reports describing the mode of action of yeast DFMs help to understand some of the observed inconsistencies reported in trials and give confirmation of the beneficial effects of DFMs in ruminants under certain feeding conditions. Amongst them, Jouany (2006) proposed a model to explain how yeast cells could interact with other rumen microbes in a 'micro-consortium structure' and improve their activity (Fig 1.1). In the proposed model Jouany (2006) highlights four main modes of action of yeast direct fed microbials.

Oxygen uptake: Yeasts cells are aerobic and have a limited ability to survive in anaerobic conditions such as the rumen. They are commonly found in small air pockets between fibres in the solid phase in the rumen and seldom found in the liquid phase (Jouany *et al.,* 1991). The scavenging of oxygen by yeast cells makes the rumen environment more anaerobic and therefore promotes the growth of anaerobic bacteria such as *Fibrobacter succinogens.* This was confirmed by authors Chaucheyras-Durant *et al*. (2008) who refer to it as oxygen sequestration. The removal of oxygen by yeast in the rumen leads to a significant decrease in the redox potential of the rumen (Jouany *et al.* 1999a, Chaucheyras- Durant and Fonty, 2002). This change improves the environment for growth of cellulolytic bacteria and stimulates there attachment to cellulose particles (Roger *et al.,* 1990). A number of researchers (Offer, 1990; Callaway and Martin, 1997; Doreau and Jouany, 1998) all reported this to increase the initial rate of cellulolysis and therefore improving voluntary feed intake. The redox potential also influences the lactate-propionate equilibrium in the rumen as explained by Marden and Bayourthe (2005). The authors showed that when the redox potential is decreased the formation of propionate is favored at the expense of lactate, therefore alleviating the fall in rumen pH associated with lactate production. This influence on the lactate-propionate equilibrium explains why the live yeast cells can regulate rumen pH while being fed diets high in concentrates which upon fermentation generate large amounts of acids especially lactic acid, which leads to rumen acidosis (Williams *et al.,*1991).

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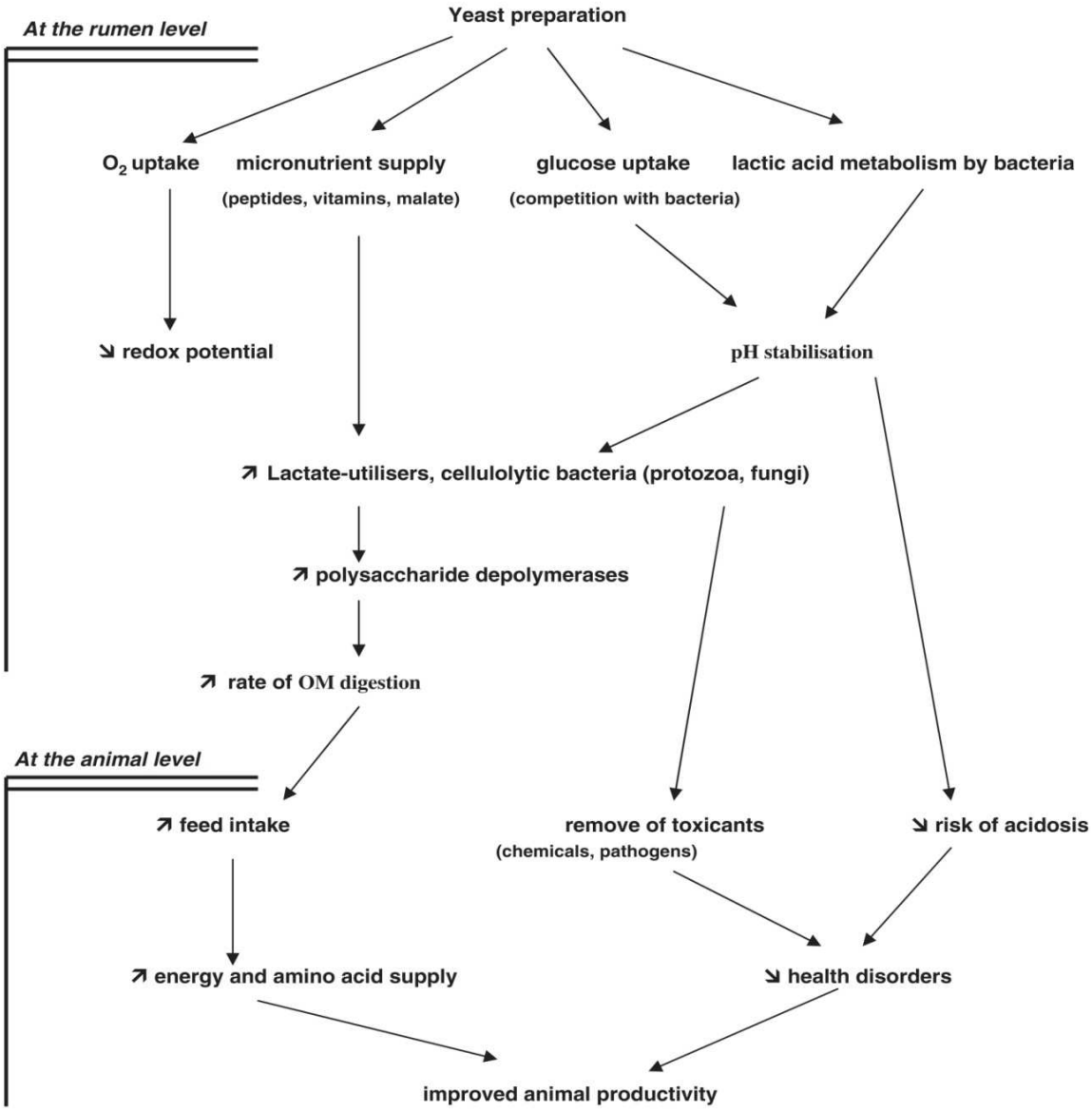

Figure 1.1.: Proposed model to describe the mode of action of yeast in the rumen and consequences for ruminants (From Jouany, 2006)

Microbial stimulation: The work done by Jouany in 2006 was initially based on work done by Wallace and Newbold (1995), where the authors suggested one of the mode of actions was through microbial stimulation. They reported a 50% increase in viable rumen bacteria; this was found in 14 studies where animals were fed *S. cerevisae*. Wiedmeier *et al.* (1987) reported that supplementation of yeast DFM's stimulated *Fibrobacter succinogens, Ruminococcus* spp. and *Butyrivibrio fibrosolvens* all of which are primarily fibre digesting bacteria. Subsequent to these observations Chaucheyras-Durand *et al.,* (1995) reported improvement in cell wall colonization by the rumen fungus *Neocallimastix frontalis.* Chaucheyras- Durand *et al.* (2005) found that this was partly due to the fact that yeast DFM provides thiamine for zoosporogenesis of the *N. frotalis.*

Glucose uptake: Chaucheyras *et al.* (1996) reported that *S. cerevisae* competes with *Selenomas ruminantium* and *Lactobacilli* spp. for glucose use; this was confirmed by Goad *et al.* (1998). Both of these organisms are primarily lactic acid producing bacteria, thereby further buffering the negative effect of lactic acid on rumen pH. Yeast supplementation can also promote an increase in rumen

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protozoa concentration (Jouany *et al.,* 1999b). Since protozoa compete with *S. bovis* for glucose uptake and are able to metabolize lactic acid, they actively play a role in lactic acid concentrations in the rumen (Chamberlain *et al.,* 1983; Newbold *et al.,* 1985)

Micronutrient supply: Yeast cells die within a few hours of entering the rumen due to their inability to survive for extended periods in an anaerobic environment. They are then autolysed and release their cell contents into the rumen. The cell contents are rich in certain favourable substrates such as malate and small peptides, which stimulate the use of lactate by rumen microbes such as *Megasheara elsdenii* and *S. ruminantium* (Callaway and Martin, 1997). This impacts favourably on modulating the rumen pH rumen.

b) Effects on ruminal N and energy utilization

It is challenging to find consistent responses, or lack thereof, when reviewing the effect of yeast DFM's on ruminal fermentation parameters. This is normally due to variance in yeast strain, diets and number of experimental animals especially in cattle. In theory stimulating the microbial populations, in the rumen, can lead to an increase in fermentation rate (Ryan and Gray, 1989; Newbold and Rode, 2006) and microbial protein synthesis. This should alter fermentation patterns and improve ruminal nitrogen utilization (Wallace and Newbold, 1995).

Rumen ammonia concentrations, although postulated to reduce upon the addition of a yeast culture or live yeast, have shown inconsistent results when supplemented to ruminants. Similar tendencies applied to the reduction of proteolytic bacterial populations in trials conducted on the above mentioned hypothesis. Reductions in proteolytic bacteria populations and/or ruminal ammonia concentration have been reported in some studies such as those conducted by Chaucheyras- Durand *et al.* (2005), Kumar *et al*. (1994) and Harrison *et al.* (1988). However Erasmus *et al.* (1992) and Putnam *et al. (*1997) reported no significant reductions in either. Many of the authors that did not report a significant reduction in ruminal ammonia concentration concluded a tendency for a decrease (Erasmus *et al.,* 1992). Erasmus *et al.* (1992) indicated the reduction of rumen ammonia to be a result of an increase in amino acid synthesis and incorporation into microbial protein.

Wallace and Newbold (1995) reported that *S. cerevisae* based yeast additives did not have a significant effect on volatile fatty acid concentrations. This is in agreement with the data presented by Chaucheyras- Durand *et al.* (1997) as well as other experiments that showed no significant effect on volatile fatty acid concentration; where significant effects occurred; authors suggested they had little biological significance. Several authors (Harrison, *et al.,* 1988; Martin, *et al.,* 1989. Williams, *et al.,* 1991), however, reported a tendency for a lower acetate : propionate ratio when yeast cultures or live yeast were supplemented in the diet. Williams and Newbold (1990) suggested that this tendency is most likely to be the result of increased production of propionate rather than reduced production of acetate.

Methane production was reported to be reduced upon the addition of yeast cultures or live yeasts in some studies (Mutsvangwa *et al.,* 1992; Lynch and Martin, 2002), however, not in others (Mathieu *et al.,* 1996; McGinn *et al.,* 2004). Differences in methane emission results can be due to numerous factors including yeast strain, duration of *in vitro* studies, age of experimental animals and experimental diet. All of these factors could possibly have prevented the yeast based additives from stimulating favourable rumen microbes (Wallace and Newbold, 1995; Newbold and Rode, 2006).

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c) Effects on animal performance and health

In beef cattle literature has been consistently published pertaining to the improvement of performance by supplementing both live yeast and yeast cultures. *S. cerevisae* additions in beef cattle have been shown to result in a 7.5% improvement in live weight gain (Garcia, 1999). This naturally depends on the diet being evaluated. Diets high in starches and sugars, such as those commonly fed in South African feedlots, have shown to have live weight improvement of up to 13% (Garcia, 1999). Newbold *et al.* (1993) reported that experiments investigating roughage sources in beef cattle diets; maize silage tended to have an improved response over grass silage with yeast supplementation.

2.3.2 Bacterial DFM

Much of the initial attention of feeding bacterial DFMs arose due to the health benefits reported in humans and monogastrics in the mid-1950s. Soon thereafter the interest in feeding preruminant calves bacterial DFM's followed. Most of the DFM's that were studied were *Lactobacillus, Entercoccus, Steptococcis* and *Bifidobacterium* (Newman and Jacques, 1995). The emphasis being on the acceleration of the colonization of bacteria (mainly *Lactobacillus* spp.) in the pre-developed rumens and intestines, the improvement of health and rapid adaption to solid feed of weak and/or stressed calves was aptly illustrated by Bechman *et al.* (1977); Maeng *et al.* (1987) and Fox (1988). Most of the focus of feeding bacterial DFMs remained on preruminants until approximately 1980 when various authors (Crawford *et al*., 1980; Hutcheson *et al*., 1980; Kiesling and Lofgreen, 1981; Davis, 1982; Kiesling *et al*., 1982; Hicks *et al*., 1986) conducted several feedlot trials to evaluate the efficacy of a combination of *Lactobacillus* spp.

a) Mode of action

Due to the great variability of the response to feeding bacterial DFM in adult ruminants the mode of action is still to be completely understood. Krehbiel *et al*. (2003) divided the probable modes of action into four groups of which three will be discussed in this section and the fourth in the following section.

Competitive attachment: Jones and Rutter (1972) reported that the attachment of certain strains of *E. coli* was crucial in order for the production of the bacteria's enterotoxin. Therefore, if *E. coli* could not attach to the intestinal wall due to the presence of attachment of bacterial DFM's there would be a reduction in enterotoxin production. Attachments of bacterial DFM's were also speculated by Salminen *et al.,* (1996) to support gut epithelial proliferation and to reduce peristaltic removal of organisms. This mode of action would naturally not only apply to *E. coli* but to other unfavourable bacterial species that require attachment to the intestinal wall.

Antibacterial effect*: L. acidophillus* in particular amongst the Lactobacillus spp. has been shown to inhibit the growth of certain pathogens such as *E. coli, Salmonella typhimurium, Staphylococcus aureus*, and *Clostridium perfringens* (Gilliland and Speck, 1977). Mann *et al.* (1980) showed that in the presence of Lactobacillus spp. lambs could tolerate infection of a very pathogenic strain of *E. coli*. This could partially be due to the hydrogen peroxide produced by lactobacilli which has an antagonistic interaction with other bacteria (Gilliland and Speck, 1977). Additional reports suggest that the antimicrobial mode of action is as a result of antimicrobial proteins and/or bacteriocins that mediate or facilitate the above mentioned antagonism (Krehbiel *et al.,* 2003).

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Immune response: It was postulated by two reviews (Erickson and Hubbard, 2000; Isolauri *et al*., 2001) that bacterial DFMs promote intestinal health and overall host health by modulation of the host immunity (Krehbiel *et al.,* 2003). Bacterial DFM's have been shown to affect mainly the innate immune system (Erickson and Hubbard, 2000; Isolauri *et al*., 2001). Supplementation of bacterial DFM generally leads to an increase in phagocytosis and natural killer cell activity, both of which are characteristic of an innate response (Erickson and Hubbard, 2000; Isolauri *et al*., 2001).

b) Effects on ruminal N and energy utilization

Nocek *et al.* (2002) reported that bacterial DFM's (notably lactate-producing bacteria) might help ruminal microorganisms adapt to the presence of lactic acid. Recent research on lactate-utilizing bacteria such as *M. elsdenii* has shown the prevention of lactate accumulation when highly fermentable carbohydrates are fed (Greening *et al.,* 1991; Kung and Hession, 1995; McDaniel *et al.,* 2009, Henning *et al.,* 2010 a; b). Inconsistent results as to the total volatile fatty acid production in the rumen have been reported. In the study by Kung and Hession (1995) it was shown that despite the improved rate of ruminal pH recovery the total VFA production in the *M. elsdenii* treated animals were more than twice that of the control. Contrary to Kung and Hession (1995), however, other authors (Aikman *et al.* 2009a; Hagg *et al.*, 2010; Henning *et al.*, 2010a) reported no difference in total volatile fatty acid concentration in the rumen. The acetate concentrations did not seem to differ between treatments and the authors reported the differences in VFA concentrations to be that of butyrate, valerate and other branched- chain fatty acids. Greening *et al.* (1991) reported that *M. elsdenii* supplemented beef cattle improved the minimum pH and reduced the lactate concentration when the cattle were experimentally induced with acidosis. *M. elsdenii* simultaneously uses lactate, glucose and maltose as an energy source and therefore competes for substrate with lactate-producing microorganisms in the rumen (Russel and Baldwin, 1978). Krehbiel *et al.* (2003) suggested to achieve the most likely benefit in energy metabolism in the rumen one must supplement a combination of *Lactobacillus* and *Propionibacterium* strains. This suggestion is due to the inhibition of methane production by lactate production by *Lactobacillus* which will in turn promote propionate production by *Propionibacterium* and therefore improve the energy efficiency of the animals.

c) Effects on animal performance and health

Ware *et al*. (1988) was one of the first authors to report that *L. acidophilus* increased daily gain and improved feed efficiency in yearling steers fed a high-concentrate diet compared with controls. Supplementation of lactate-producing and/or lactate-utilizing bacteria has in recent times shown to improve feed efficiency and daily gain of feedlot cattle (Swinney-Floyd *et al*., 1999a; Galyean *et al*., 2000; Rust *et al*., 2000a, b). A meta-analysis done by Krehbiel *et al.* (2003) reported that the results from six experiments suggest that feeding bacterial DFM to feedlot cattle results in approximately 2.5% to 5% increase in ADG and an approximately 2% improvement in FCR, whereas DMI data was inconsistent.

Bacterial DFM have been shown to decrease morbidity in newly weaned/received calves. As discussed previously in this section, certain strains of bacterial DFM can alleviate rapid drops in ruminal pH, therefore reducing the incidence of sub-clinical and clinical acidosis. McDaniel *et al.* (2009) and Henning *et al.* (2010a; b) showed a decrease in ruminal lactate concentrations in response to *M. elsdenii* treatment as well as a significantly higher ruminal pH post inoculation via rumen cannula. Improvement of immune modulation as discussed previously has been reported, improving the animal's ability to combat pathogenic challenge. *L. acidophilus* has also been shown to reduce faecal *E. coli* O157:H7 and coliform shedding by feedlot cattle (Ohya *et al.,* 2000).

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From numerous reports and studies it can be ascertained that ionophores, antibiotic growth promoters and direct fed microbials have been shown to positively influence feed efficiency, by improvement of energy and/or ruminal N utilization. Positive effects on animal performance and health can also be attributed to these additives. The magnitude of the response to the inclusion of rumen modifiers varies significantly. The effect of the type of diet has been shown to play a large role in the response to rumen modifiers. Therefore it is of immense importance that all rumen modifiers be studied under local conditions with diets that are representative of the locally fed diets.

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Chapter 3

Effect of a natural fermentation liquid supplement on health, performance and carcass characteristics of beef feedlot cattle

1. Introduction

Lactobacillus Fermentation Prototype (LFP; Diamond V, Cedar Rapids, Iowa) is a recently developed all natural fermentation liquid from Diamond V. It is manufactured using a proprietary anaerobic bio-fermentation of *Lactobacillus acidophilis*. It has been developed as a "prototype" that provides nutritional support for digestive efficiency and rumen health in feedlot cattle fed a diet high in concentrate. Very little research has been carried out on the product and as a result only limited data is available as to the physiological and digestive mechanism of the fermentation liquid. Promising results were obtained in a feedlot trial on a large commercial feedlot in Texas, U.S.A. (M. Scott, p*ersonal communication*, Diamond V, Cedar Rapids, Iowa, 2011*)*. Despite admission being restricted only to the finisher diets results showed that beef cattle fed the fermentation liquid had gained 9% more body weight and a 6% increase in feed efficiency was achieved, compared to the control. No research has been done on the LFP in South Africa where the feeding period is significantly shorter than that of the U.S.A. Furthermore there are differences in both the diets fed and the carcass grading systems in the two countries. In South Africa, unlike in the U.S.A, one of the primary energy sources in feedlot diets is hominy chop, which is variable in quality especially the fat content. Because of limited research information and the differences in feedlotting between U.S.A and South Africa, it was decided to investigate the potential of LFP liquid under typical South African conditions. The aim of this research project is to determine the effects of various levels of LFP on the performance of feedlot cattle. The parameters to be measured are growth performance (average daily gain, average daily feed intake and feed conversion ratio), health (morbidity and mortality), carcass characteristics (quality and yield), liver and rumen scores.

2. Materials and methods

2.1. Location

The study was conducted at Chalmar Beef, a commercial feedlot with a standing capacity of 25000 cattle. Chalmar Beef was established by Mr Wimpie Wethmar in 1969 making it one of the first commercial feedlots in South Africa. The feedlot is situated approximately 8 kilometers north west of Bapsfontein, (GPS coordinates: 25º56'31.08"S, 28º23'27.67"E), at an altitude of approximately 1600 meters above sea level. The climatic conditions are typical to the Highveld region of South Africa. The average rainfall is 715 mm per annum; with the majority of the rainfall occurring between October and March. The monthly distribution of average daily maximum temperatures for the Bapsfontein region; range from 16.8 °C in June to 26 °C in January. The region is the coldest during July when the temperature drops to 1.1 °C on average during the night.

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2.2. Housing and processing

A homogenous group of 240 single-sourced, crossbred beef steers were be used for the trial. Inspection for uniformity across frame size, body weight (215- 265 kg) and body condition was carried out upon arrival of steers.

Upon arrival at the feedlot the steers were rested; where they received hay and water *ad lib,* and then sorted. Steers were individually weighed, tagged and then vaccinated against clostridial and viral threats (BoviShield® Gold, Maxitet® LA, Multisomni) as well as treatment for internal and external parasites (Ivomec® Super, Blitzdip®). Injured or non-conformative steers were culled from the group. The steers were placed on free range pasture for 8 days. After 8 days booster vaccinations were administered (BoviShield®, Covexin®, Micotil®, Botuthrax®, Multisomni), Ralgro® implant, castration and dehorning were also carried out. The steers were housed in outdoor pens (50mx3m) with an overhead roof over the feedbunk.

2.3. Treatment and blocking of animals

The experimental design followed was a randomized complete block design with three treatments. Based on processing weights and withdrawals one hundred and forty four steers were selected for blocking according to weight. Steers were then blocked into three groups of 48 animals each. Within each group steers were randomly allocated to one of eight pens with each pen holding six steers. This resulted in a total of 24 pens (8 pens per treatment, 6 steers per pen). With the layout of random assignment of pen within block and replicate. The experimental treatments were as follows:

1) Control: 0 g LFP liquid/Animal/Day
2) LFP-5: 5 g LFP liquid/Animal/Day
3) LFP-10: 10 g LFP liquid/Animal/Day

2.4. Diet and feeding

Three diets were used for the duration of the trial, starter (0-21days), grower (22-35 days) and finisher (day 36- completion). The ingredient composition of each diet is indicated in Table 3.1. A total mixed ration was prepared tri-weekly at Chalmar Beef, Bapsfontein, and bagged for manual feeding during the trial. Bunk management was done identical to the commercial application at Chalmar Beef to a) simulate commercial conditions and b) to replicate *ad lib* feed intake whilst offering fresh feed twice a day. Feeding took place twice daily (at 07h00 and 12h00). Diets were identical (including feed additives) within each replicate with the exception of the above mentioned treatments. The LFP liquid was administered into the feed at the required inclusion level estimated by average DMI over the previous 3 days and mixed thoroughly. LFP administration was done by spraying the LFP liquid onto the feed in the feed mixer during the mixing process. Daily feed offered and weekly orts removed were recorded, sampled and frozen for analysis of nutrient intake and to establish weekly dry matter feed intake. Fresh clean water was available to the steers at all times during the trial. The inclusions of Zinc Methionine (ZinMet®, Global Animal Products), ENS Gel Composite (Essential Nutrient Systems) and Tylan 100® (Elanco Animal Health) were to replicate the commercially included additives in feedlots in South Africa

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Table 3.1 Ingredient composition of the three diets fed during the different growth periods

	Starter	Grower	Finisher
	DM %	DM %	DM %
Ingredient			
Yellow Maize	-	22.82	23.97
DFG[1]	25.09	-	-
Wheaten Bran	40.86	28.77	1.65
Hominy Chop	-	16.40	48.64
Whole Maize Plant (Chopped)	29.00	29.32	19.57
Soyabean Oilcake	2.97	-	2.67
Limestone	0.990	1.42	1.90
Salt	0.780	0.190	0.253
Urea	0.240	0.721	0.914
Ammonium Sulphate	-	0.289	0.366
Rumensin	0.010	0.014	0.016
Starter Vitamin & Mineral Premix	0.060	-	-
Grower Vitamin & Mineral Premix	-	0.011	0.012
Zinc Methionine 20% [2]	-	0.010	0.010
ENS Gel Composite[3]	-	0.027	0.031
Tylan 100[4]	-	0.009	0.010
Total	**100.00**	**100.00**	**100.00**

[1]Defatted Germ (Ruto Mills)

[2]Zinc Methionine (ZinMet 20%® Global Animal Products)

[3]ENS Gel Composite (Essential Nutrient Systems, South Africa)

[4]Tylan 100 (Elanco Animal Health)

$$\textbf{LFP inclusion (kg)} = \left[\frac{\left(\frac{\text{LFP treatment (gram/head/day)}^{1}}{1000} \right)}{\text{Average DM intake (3days) (kg)}} \right] \text{x Batch DM (kg)}$$

[1] 5 grams/head/day (LFP-5) or 10 grams/head/day (LFP-10).

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2.4. Feed sampling

Feed sampling was carried out as follows: An equal amount of untouched feed was collected by taking grab samples at different places in the feed bunk from each pen and then pooled according to treatment, resulting in three representative feed samples per feeding period. Representative sub-samples of each were then taken using the manual cone and quartering method (Faithfull, 2003). During the starter and grower diet feeding periods, samples were taken for each batch of feed that was mixed and then pooled and sub-sampled for each feeding period, whereas during the finisher diet feeding period samples were pooled and sub-sampled every 40 days. Feed samples were analysed at the UP Nutrilab (University of Pretoria, Pretoria, Gauteng) according to the procedures of the AOAC (2000) and Van Soest *et al.* (1997).

2.5. Implanting and interim weights

All the steers received a zeranol implant (Ralgro Super®, Intervet South Africa, Isando, South Africa) on day 9 of the trial and then reimplanted with a TBA-estradiol benzoate (Revalor®, Intervet South Africa, Isando, South Africa) on day 37 of the trial. This was done to simulate the normal procedure in South African feedlots. Steers were weighed and recorded prior to receiving the implants.

2.6. Morbidities and mortalities

Steers that exhibited any respiratory sickness were pulled. If only for the first time then they were treated with Baytril® (Bayer Animal Health, Isando, South Africa) followed by a day 3 Procapen® (Bayer Animal Health, Isando, South Africa) treatment and immediately returned back to their pen. Steers pulled for a second respiratory treatment were treated with Nuflor® (MSD Animal Health, Kempton Park, South Africa) and Finadyne® (MSD Animal Health, Kempton Park, South Africa). Steers that exhibit non-respiratory sickness were pulled, treated and returned pen, consecutive treatments were administered if and until reasoned to be necessary. Chronically ill steers were weighed and removed from the pen without being re-entered. All pulls, treatments, returns, deaths and cause of deaths were recorded along with individual tag number and date thereof. Dead animals were weighed before disposal.

2.7. Slaughtering and carcass data

The steers were marketed at the discretion of the feedlot manager (when the steers were deemed adequate for slaughter). The duration of the trial was 134 days, 8 days free range pasture and 126 days in the feedlot. All steers were weighed and then sent to the adjacent Chalmar Beef's abattoir where they were slaughtered and carcass data obtained.

2.8. Schedule of trial events

Day 0: Cattle arrive at feedlot.
Day 1: Cattle are individually weighed and receive all vaccinations.
Day 9: Cattle are individually weighed and receive vaccination boosters and implant.
Day 21: Diet change – starter → grower.
Day 37: Cattle are individually weighed and received re-implant (diet change) grower → finisher.
Day 78: Cattle are individually weighed.
Day 106: Cattle are individually weighed.
Day 134: Cattle are individually weighed and slaughtered at the abattoir.

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2.9. Parameters measured during the trial

The following parameters were monitored:

Cattle

- Performance data
  - Individual body weight (BW) (days: 1, 9, 37, 78, 106, 134)
  - Individual average daily gain (ADG) (per period and overall)
  - Pen average daily feed intake (ADFI) (per period and overall)
  - Pen feed conversion ratio (FCR) (per period and overall)
- Health data
  - First time pulls (% per period and overall)
  - Subsequent pulls (% per period and overall)
  - Chronic pulls (% per period and overall)
  - Mortality (% per period and overall)
  - Weight of dead steers
- Carcass data
  - Hot carcass weight
  - Cold carcass weight
  - Dressing percentage
  - Carcass yield and grade
  - Rumen and liver scoring

Diets

- Feed composition
- Feed cost
- Daily feed offered
- Weekly orts removed
- Feed sample analysis of starter, grower and finisher diets
  - Dry matter (DM)
  - Crude protein (CP)
  - Acid detergent fibre (ADF)
  - Neutral detergent fibre (NDF)
  - Non-fibre carbohydrates (NFC) calculated as follows: NSC=100-(%CP+%NDF+%EE+%Ash) (NRC, 2001)
  - *In vitro* organic matter digestibility (IVOMD)
  - Gross energy (GE)
  - Ether extract (EE)
  - Ash
  - Calcium
  - Phosphorus
  - Calculated Metabolizable Energy [ME=0.82x(IVOMD x GE)] (Robinson *et al.,* 2004)
- Orts sample analysis
  - DM (Dry matter)

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2.10. Sample analyses

Laboratory analyses were done at Nutrilab, Department of Animal and Wildlife Sciences, University of Pretoria. Feed samples were analyzed in duplicate for DM (procedure 934.01 AOAC, 2000), CP was analyzed using Leco analysis (procedure 968.06 AOAC, 2000), EE (procedure 920.39 AOAC, 2000), ADF (Goering & Van Soest, 1988), NDF (Van Soest & Robertson, 1997), GE (ASTM D2015), ash (procedure 942.05 AOAC, 2000), calcium (Giron, 1973) and phosphorus (procedure 965.17 AOAC, 2000).

2.11. Statistical analysis

The data was analyzed as a randomized complete block design using Proc mixed procedures of SAS (Statistical Analysis Systems, 2011) with pen as the experimental unit. The model is shown below:

$$y_{ij} = \mu + T_i + B_j + \varepsilon_{ij}$$

Where y_{ij} is the response due to the experimental unit, μ is the overall mean, T_i is the treatment effects, B_j is the random effect associated with the j^{th} block and ε_{ijk} is the random error associated with the experimental unit j that received treatment i. Assumptions for random effects were block effects and were distributed normally and independently; errors were distributed normally and independently. Standard errors of the mean (SEM) were calculated. Significance was declared at $P<0.05$ and tendencies at $P<0.10$ as determined by the Fisher test (Samuels, 1989). Standard chi-square tests were used for the morbidity data, and the data was analyzed with the frequency model of SAS (2011). Once again the level statistical significance was $P<0.05$.

3. Results and discussion

3.1. Chemical composition of experimental diets

Pooled samples of the 12 experimental diets used in the experiment were analyzed and the chemical composition of each period and treatment diet is shown in Table 3.2.

The variation between the compositions of the treatments were reasonably high, this could partially be explained by the large variation in the composition of the whole chopped maize plant that was used. The variation is in part due to the fact that the ingredient is transported for a relative distance on a poor surface road leading to the separation of the chopped stalk, leaves and grain from one another. Additionally due to practical constraints the whole chopped maize plant was loaded into the feed mixer with a front-end loader further increasing the possibility of discrepancies occurring. Despite great care being taken during the trial period sampling errors and mixing inaccuracy may also have contributed to the variation.

It must be noted that the inclusions of these relatively high levels of whole maize plant are not common in commercial feedlots in South Africa. The trial diets had, unfortunately, to be adapted to accommodate the general mixing and feeding practices at Chalmar Beef. These were that the feed could not be prepared daily, thus the inclusion of the more common ingredient, maize silage, had to be omitted completely so as to ensure the feed did not spoil either prior to feeding or in the feed bunks.

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Table 3.2 Chemical composition (%DM) of the 12 experimental diets fed during the trial (3 LFP treatments[1], with 3 feeding periods within each treatment)

	Diets (%DM)											
	Starter			Grower			Finisher I			Finisher II		
Chemical Composition	Control	5 g	10 g	Control	5 g	10 g	Control	5 g	10 g	Control	5 g	10 g
DM (%)	86.36	86.91	87.08	85.84	86.08	86.16	85.87	85.34	85.43	85.53	85.35	86.23
Crude Protein (%DM)	14.80	14.69	15.09	13.25	12.37	12.74	12.99	13.21	13.17	11.81	11.60	12.08
GE (MJ/kg)	17.79	17.64	17.48	18.06	18.09	18.01	17.96	17.96	17.98	17.94	18.16	17.94
ME(MJ/kg)[2]	11.21	11.25	11.10	11.29	10.75	11.55	11.38	11.56	11.77	11.76	12.17	12.10
NDF (%DM)	34.74	35.26	36.39	29.95	31.89	30.38	24.67	24.35	23.96	23.45	23.62	23.23
ADF (%DM)	12.80	14.06	13.70	11.66	12.60	11.57	8.81	9.30	8.37	8.29	9.19	9.23
EE (%DM)[3]	2.75	2.62	2.72	3.56	3.57	3.57	6.20	6.31	6.26	6.28	6.24	6.30
NFC (%DM)[4]	43.21	42.84	40.86	49.98	48.41	49.27	52.38	52.54	53.24	54.52	54.72	54.08
IVOMD (%DM)[5]	76.82	77.73	77.44	76.27	72.45	78.21	77.23	78.46	79.82	79.94	81.76	82.23
Ash (%DM)	4.51	4.59	4.94	3.27	3.77	4.05	3.76	3.59	3.37	3.94	3.82	3.92
Ca (%DM)	0.81	0.77	0.78	0.67	1.10	0.95	0.61	0.65	0.57	0.61	0.53	0.57
P (%DM)	0.66	0.68	0.98	0.49	0.47	0.47	0.31	0.32	0.33	0.29	0.29	0.30

[1]LFP Treatments(Control: 0 g LFP/head/day, 5 g: 5 g LFP/head/day, 10 g: 10g LFP/head/day)

[2]Metabolable Energy(MJ/kg DM) = 0.82 x (GE x IVOMD)/100 (Robinson *et al*., 2004)

[3](EE) Ether Extract

[4]Non-Fiber Carbohydrates (%DM) = 100 - (CP + NDF + EE + Ash) (NRC, 2001)

[5](IVOMD) In-Vitro Organic Matter Digestibility

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3.2. The effect of LFP supplementation on the performance parameters of steers

3.2.1. Introduction

There is no published data available on the effect of LFP on the performance of feedlot steers and therefore it is difficult to discuss and compare results to other studies. In the following sections results will be compared to other results obtained from feedlot studies with supplemented with DFM's, ionophores and antibiotic growth promoters where applicable.

3.2.2. Body weight gain

Body weight gain from day 0 to slaughter (day 134) is shown in Table 3.3. Despite blocking by live mass occurring on day 1 all the steers retained a mean body weight gain of 8 kg for the period on the pasture. Upon commencement of the trial the steers had a mean live body weight of 241 kg, 240 kg, and 241 kg for the control, 5 g /head /day and 10 g /head /day treatment respectively. At the conclusion of the feeding period the mean live body weight of the control, 5g LFP and 10g LFP treatments were 459 kg, 462 kg and 461 kg respectively and did not differ (P>0.05). Live body weight performance indicates that there was no distinct advantage for the supplementation of LFP, at both treatment levels; thus contradicting previous observations of a 9% increase in body weight gain (M. Scott, *personal communication,* Diamond V, Cedar Rapids, Iowa, 2011)

Table 3.3 Growth performance (body weight gain) of steers fed different levels of LFP

	Treatment[1]				*P*-value
	0	**5**	**10**	**SEM**	**Treatment**
Body Weight, kg[2]					
Arrival	233	232	233	0.83	0.57
Day 9	241	240	241	0.83	0.57
Day 37	280	281	280	2.40	0.88
Day 78	363	361	363	4.09	0.89
Day 106	421	418	421	4.71	0.88
Final	459	462	461	5.08	0.95

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

[2]Arrival= Day 1 weight when cattle arrived and were put on pasture; Day 9 = Weight when cattle were allocated to experimental pen from pasture; Day 37 = weight at time of re-implant; Final = day 134 weight.

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3.2.3. Average daily gain (ADG)

Average daily gain (ADG) was measured and recorded for the interim weighing periods as well as the total feeding period. As shown in Table 3.4, the ADG in the first period (day 9 to day 36) was 1.40 kg, 1.49 kg and 1.39 kg for control, LFP-5 and LFP-10 treatment group of steers respectively. There was, however, no statistical difference in ADG in the period from day 9 to day 36 between the treatment groups (P=0.62). There was also no difference (P=0.58) reported from day 37 to day 77 between control, LFP-5 and LFP-10 (2.04 vs 1.94 vs 2.02). Neither was there any differences observed in the subsequent 2 periods, from day 78 to day 106 and from day 107 to day 134 (P>0.05). The ADG over the whole period shows no difference in ADG between treatments (P=0.90).

The average daily gain achieved by all the steers in this experiment could be considered representative for South African feedlots during the time the experiment was conducted (Esterhuizen *et al.,* 2008; Haasbroek, 2013). Haasbroek (2013) reported an average ADG of 1.83 kg as opposed to a slightly lower ADG of 1.76 kg reported in this trial. It could possibly be noted that considering the lowered palatability of the diet due to the inclusion and/or omission of the whole chopped maize plant and silage, respectively, the steers achieved satisfactory average daily gain. The exception however would be the final feeding period (day 107 to day 134) where a 32%, 23% and 32% reduction in ADG occurred. No noticeable environmental or management anomalies were recorded during this period. Figure 3.2 aptly illustrates this unusual drop in ADG during the final finisher period and it will be discussed later in this chapter.

The performance results of this experiment suggest supplementation of LFP has no effect on average daily gain. Consideration, however, needs to be taken for the final feeding period where it is hypothesized that an external factor was limiting the body weight gain of the steers. Further experiments, possibly with a larger number of animals, will need to be conducted to confirm or contradict this inconclusive observation.

Table 3.4 Growth performance of steers (average daily gain) fed different levels of LFP

	Treatment[1]			***P*-value**	
	0	**5**	**10**	**SEM**	**Treatment**
ADG, kg					
Day 9 to 36	1.40	1.49	1.39	0.08	0.62
Day 37 to 77	2.04	1.94	2.02	0.07	0.58
Day 78 to 106	2.05	2.05	2.09	0.05	0.80
Day 107 to 134	1.39	1.57	1.42	0.06	0.11
Day 9 to 134	1.75	1.78	1.76	0.04	0.90

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

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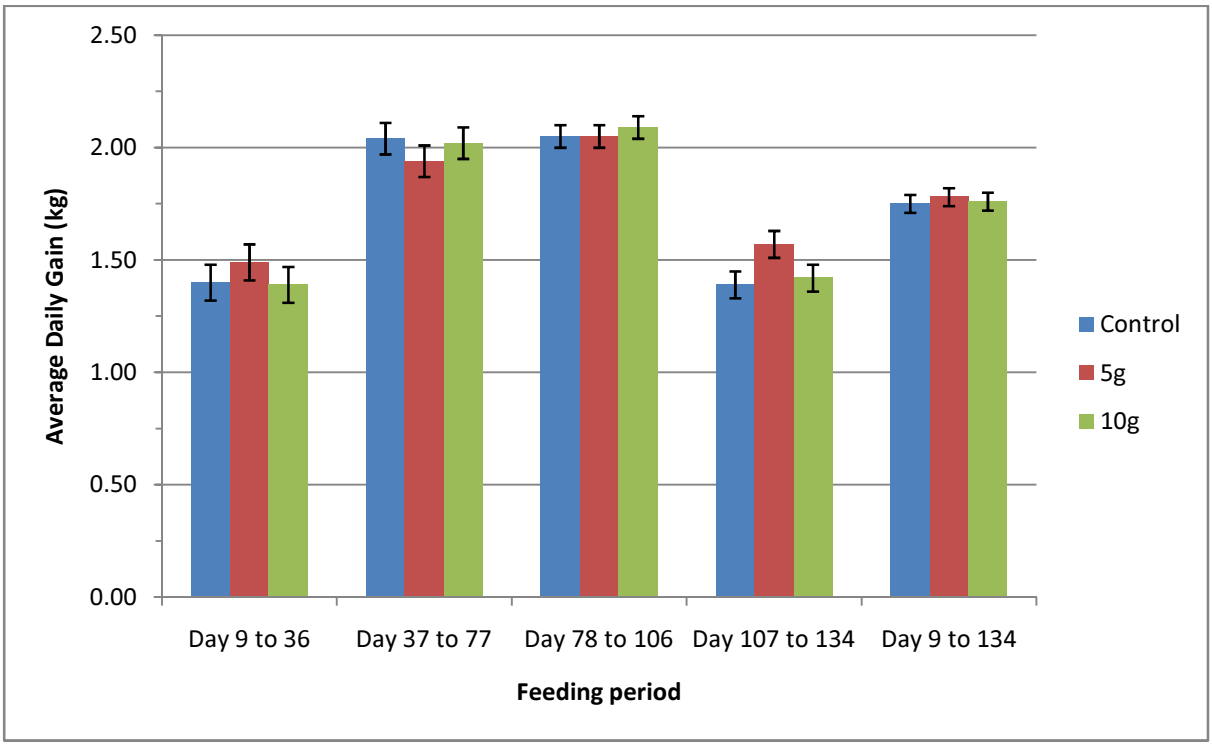

Figure 3.1 Growth performance of steers (n=144) (average daily gain) fed different levels of LFP

3.2.4. Dry matter intake (DMI)

Daily feed offered and weekly orts removed were recorded, average daily DMI was calculated for the same periods used when calculating mean body weight. Table 3.5 depicts no significant differences in DMI between the treatment groups in this experiment. Figure 3.3 clearly shows that the observed reduction in ADG was not as a result of feed restriction; the DMI for the period day 107 to day 134 was acceptable and is comparable to other feedlot studies (Esterhuizen *et al.,* 2008, Haasbroek, 2013). Esterhuizen *et al.* (2008) reported a DMI of 8.90 kg, Haasbroek (2013) an average DMI of 8.67 kg and in this experiment an average DMI of 8.64 kg; with all three treatments being within the range reported by Esterhuizen *et al.* (2008) and Haasbroek (2013). This supports the hypothesis by the author that an environmental factor influenced the live weight gain of the experimental animals. It should be noted that in contrast to the DMI commonly observed in commercial feedlots in South Africa, the dry matter intakes of this experiment are slightly lower than what would be observed commercially. This could partially be explained by three factors. Namely the reduced palatability of the diets due to the omission of the maize silage and inclusion of higher levels of whole chopped maize plant. Secondly the unconventional pen design (50 m x 3 m) resulted in a relatively small feed bunk space (0.5 m per animal) to pen size ratio which could have influenced feeding behavior. Thirdly the feed bunk management practices vary significantly from feedlot to feedlot. The latter is of significant value considering that cattle can very easily select out the stalk component of the whole maize plant when it is dry, as in this case, and this could have lead to restricted feeding as the bunks were judged to have adequate feed quantities, yet the residue feed was mainly composed of relatively unpalatable maize stalks. It was observed that feed selection did occur in this experiment, visually the remaining component were mainly chopped maize stalks.

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Results from this study suggests LFP supplementation both at 5 grams/head/day and 10 grams/head/day did not influence dry matter intake of cattle under common South African conditions.

Table 3.5 Effect of different levels of LFP supplementation on mean DMI of feedlot steers

	Treatment[1]			*P*-value	
	0	**5**	**10**	**SEM**	**Treatment**
Daily DMI, kg					
Day 9 to 36	5.37	5.18	5.32	0.16	0.68
Day 37 to 77	8.70	8.46	8.76	0.22	0.60
Day 78 to 106	9.82	9.57	9.97	0.26	0.55
Day 107 to 134	9.73	10.18	10.07	0.25	0.43
Day 9 to 134	8.44	8.36	8.56	0.18	0.74

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

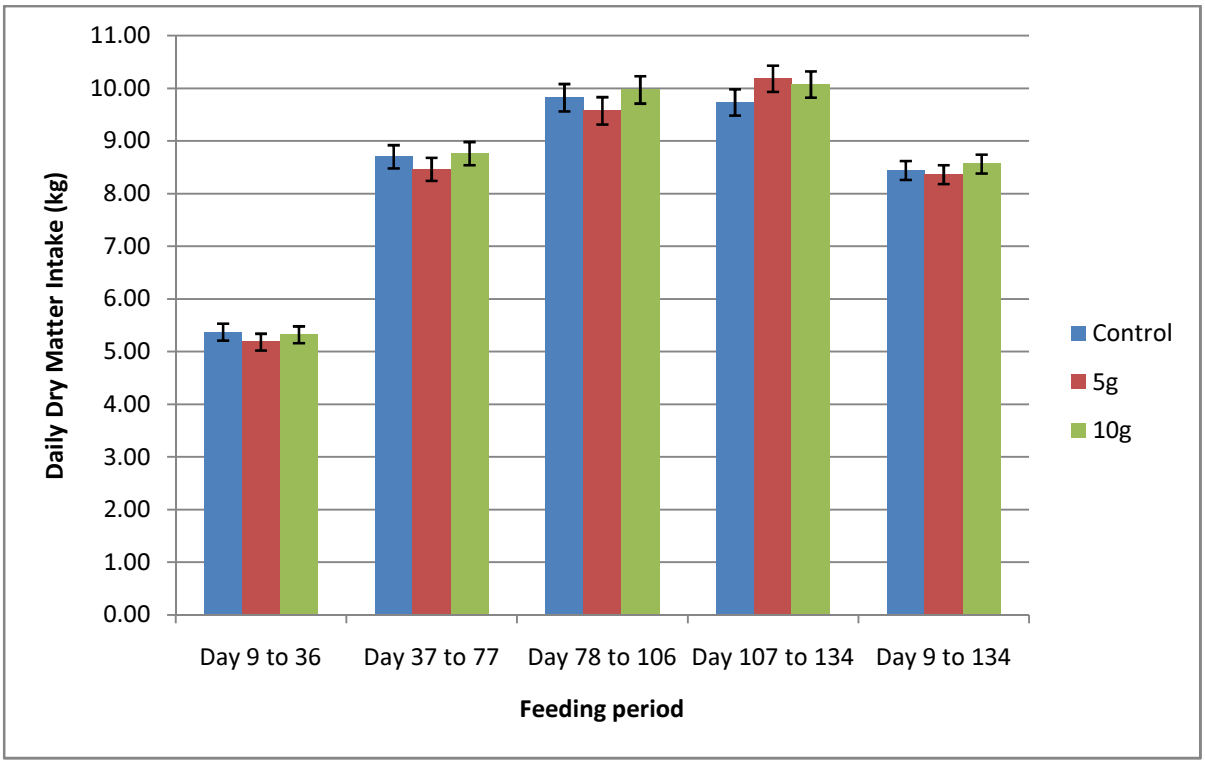

Figure 3.2 Effect of different levels of LFP supplementation on mean DMI of feedlot steers (n=144)

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3.2.5. Feed efficiency

Feed efficiency is often measured as a feed conversion ratio or feed to gain ratio. The feed conversion ratio (FCR) for the treatment groups of 144 steers in this experiment are depicted below in Table 3.6. Feed conversion ratios reported in this experiment are comparable to that of Haasbroek (2013), (Average FCR of 4.73 versus 4.79 in this experiment). As expected the FCR are the best during the first feeding phases and gradually become less efficient as the animals get older. The data shows that there is no difference (P> 0.05) in FCR between LFP-5, control and LFP-10 fed steers. There was, however, a significant improvement (P=0.03) in FCR for the LFP-5 steers when considering the entire feeding period (day 9 to day 134). Steers supplemented with LFP-5 achieved a FCR of 4.70 as opposed to the less efficient control animals and LFP-10 supplemented steers that achieved 4.82 and 4.86 respectively.

The results from this experiment suggest that LFP has the potential to improve feed efficiency of beef cattle under typical South African conditions and that the effect might be dose dependent. In previous trials improvements in FCR of up to 6% were reported over the control (M. Scott, *personal communication*, Diamond V, Cedar Rapids, Iowa, 2011). In this experiment only a 2.5% improvement in FCR was recorded.

Table 3.6 Growth performance of steers (feed conversion ration) fed different levels of LFP

	Treatment[1]			***P*-value**	
	0	**5**	**10**	**SEM**	**Treatment**
FCR, DMI (kg)/ BWG (kg)					
Day 9 to 36	3.97	3.53	3.86	0.17	0.21
Day 37 to 77	4.29	4.40	4.34	0.10	0.76
Day 78 to 106	4.79	4.66	4.78	0.11	0.66
Day 107 to 134	7.03	6.54	7.14	0.22	0.16
Day 9 to 134	4.82[a]	4.70[b]	4.86[a]	0.04	0.03

[1] Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

[ab] Row means with the same superscript do not differ significantly (P>0.05)

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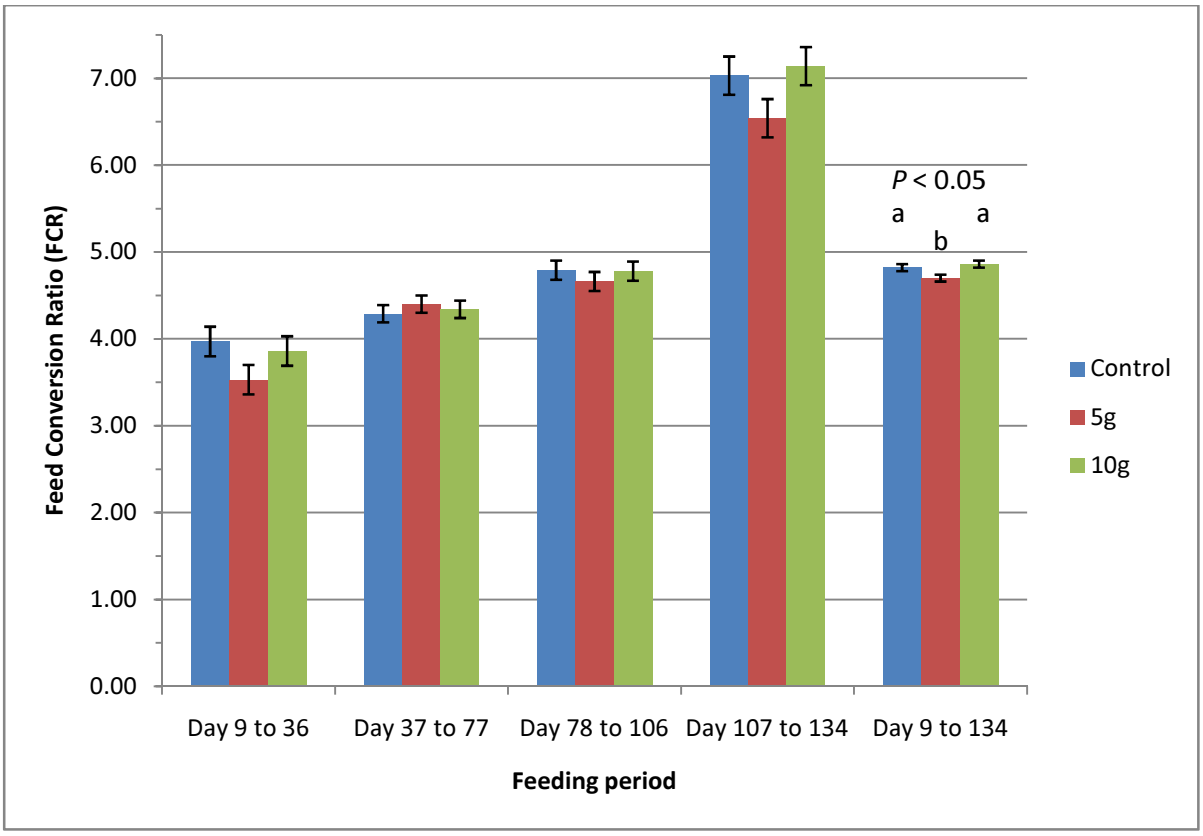

Figure 3.3 Growth performance of steers (n=144) (feed conversion ratio) fed different levels of LFP

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3.3. The effect of LFP supplementation on the health parameters of steers

3.3.1. Morbidity and mortality

The most important infectious diseases in South African feedlots are bovine respiratory disease (BRD), infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea (BVD) type 1 and 2, parainfluenza 3 (PI3), botulism, anthrax, as well as certain clostridial diseases including C. *sordellii* and C. *perfringens* type A. All of which are vaccinated against upon arrival at the feedlot. Incidences of these diseases, however, do still occur; especially in stressed calves. During winter months in South Africa bovine respiratory disease in feedlots is the most prevalent. It was reported that 42.8% of all animals had lung lesions at slaughter, but 69.5% of them had never been treated for bovine respiratory disease (Thompson *et al*., 2006). The most common metabolic diseases include rumen acidosis, bloat, laminitis and liver abscesses. These are controlled by correct nutrition, in feed antibiotics and feed bunk management.

The morbidity data was recorded and is shown in Table 3.7. Of the total of 13 pulls in the control treatment two were for minor injuries sustained, either during processing or during the feeding period, the remaining eleven pulls were for bovine respiratory illness. Only one steer was pulled for a minor processing injury in LFP-5 group and treated as per the protocol with Finadyne® whereas eleven were pulled and treated for a respiratory ailment. All steers treated in LFP-10 fed steers were pulled and treatment for respiratory illness. There was only one steer that was pulled more than once; it occurred in the control group and upon being pulled the third time was diagnosed as a chronic pull (bovine respiratory disease) on day 80, it was weighed and removed as per the protocol for treating chronic pulls. The percentage pulls for the entire period were 27%, 25% and 31% for control, LFP-5 and LFP-10, respectively. These are relatively high for a commercial feedlot even, as in this case, during winter months. Haasbroek (2013) reported an average of 15.6% incidence of bovine respiratory disease during a small pen study done on 180 animals in common South African feedlot conditions. This higher incidence of morbidity could be due to the over observation that often occurs in experiments of this nature, the number of pulls tend to be significantly higher than under standard commercial conditions with only one or two observations per day. Trial animals tend to be pulled and treated both earlier, and more rapidly than those in the commercial pens.

Only one mortality occur during the entire feeding period, this occurred in the LFP-10 fed group and was as a result of the steer needing to be slaughtered due to laminitis.

The morbidity and mortality data from this experiment does not indicate any advantage (P>0.05) of feeding both 5g/head/day LFP or 10g/head/day LFP over the control. However it must be considered that there were not any pulls and/or treatments for a digestive or metabolic disorder, as mentioned previously all pulls and treatments were for respiratory illness or a sustained injury.

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Table 3.7 The number of pulls as an indicator of the health status of steers fed different levels of LFP

	Treatment[1]		
	0	**5**	**10**
Number of Pulls			
Day 9 to 36	5	4	5
Day 37 to 77	3	3	4
Day 78 to 106	3	2	3
Day 107 to 134	2	3	3
Day 9 to 134	13	12	15

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

A standard chi-square test on the frequency of pulls during the 4 feeding periods, shown in Table 3.7, as an indicator for health status reported no difference between the 3 groups of steers, control, 5 g/head/day and 10 g/head/day ($\chi^2 = 0.5185$, *d.f.* = 6, $P = 0.9976$). There was also no difference ($P>0.05$) in the cumulative number of pulls between the control fed steers and the 2 treatment (LFP-5 and LFP-10) fed steers ($\chi^2 = 0.3500$, *d.f.* = 2, $P = 0.8395$).

3.4. The effect of LFP supplementation on the carcass parameters of steers

3.4.1. Carcass weights

Recorded hot and cold carcass weights are shown in Table 3.8. The mean cold carcass weights, 265.3 kg, of the steers slaughtered in this study were representative of common South Africa carcasses. Haasbroek (2013) recorded cold carcass weights of 258.0 kg and 245.3 kg in a small pen study and commercial trial respectively. Esterhuizen (2008) reported slightly lower cold carcass weights of 249.0 kg for feedlot cattle; they were, however, fed for a shorter time period. In this experiment both the hot and cold carcass weights that were recorded indicated that there is no advantage ($P>0.05$) of feeding LFP both at 5 g/head/day and 10 g/head/day over the control.

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Table 3.8 Slaughter data of steers fed different levels of LFP

	Treatment[1]			*P*-value	
	0	**5**	**10**	**SEM**	**Treatment**
Hot carcass weight (kg)	268.2	270.1	269.6	5.02	0.94
Cold carcass weight (kg)	264.2	266.0	265.6	4.10	0.89
Dressing percentage (%)	57.56	57.58	57.61	4.71	0.87

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

3.4.2. Dressing percentage

Dressing percentage was calculated with cold carcass weights as a percentage of final body weights of the steers. The dressing percentages achieved, (mean 57.6%), in this experiment are depictive of the common dressing percentages achieved by cattle that are not fed a ß-agonist in South Africa. ß -agonist fed cattle tend to have a higher dressing percentage, Esterhuizen (2008) reported a dressing percentage of South African feedlot cattle of 60.09%. Haasbroek (2013) reported mean dressing percentages of 57.8% and 58.0% in a commercial and small pen study respectively. The results from this experiment, shown in Table 3.8, suggest there is no effect ($P>0.05$) of LFP supplementation at either 5 g/head/day or 10 g/head/day on dressing percentage.

3.4.3. Carcass characteristics

Table 3.9 shows the carcass grading scores A2, A3 or A4 as a percentage of total carcasses graded. More than 50% of the carcasses in all three of the treatments achieved an A2 grade. LFP-5 treatment group did not have any steers that achieved a grade of A4. From the carcass characteristic data of this experiment there seems to be no impact ($P>0.05$) on carcass yield grade or quality grade of the carcasses.

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Table 3.9 Carcass grades of steers fed different levels of LFP

	Treatment[1]			*P*-value	
	0	**5**	**10**	**SEM**	**Treatment**
Percentage of Total (%)					
A2	53.00	53.20	52.70	0.83	0.99
A3	45.30	46.80	44.20	0.50	0.37
A4	1.70	0.00	3.10	0.22	0.18
Total	100.00	100.00	100.00		

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

3.4.4. Rumen and liver scoring.

Rumen and liver scoring were conducted upon slaughter of the animals. The rumen scoring data; only one rumen lesion was observed for all 142 slaughtered animals. The lesion occurred on a steer in the LFP-10 fed group of steers. The lesion was, however, given a score of 1 (rumen scoring scale of 0-5, 0 being no lesion and 5 indicating that the rumen badly damaged by severe lesions). This low incidence of rumen damage does not agree with rumen scoring data reported by Haasbroek (2013). The author reported that a mean of 49.1% of the rumens recorded were damaged. It was concluded that LFP had no noticeable effect on the clinical rumen health of steers fed either 5 g/head/day or 10 g/head/day.

The liver scoring data was deemed invalid as there was too much variation of liver grading and/or condemning from the two state inspectors earlier on the slaughtering process from where the liver scoring was being conducted. Once again negligible observations on total number of liver abscesses and condemnations were reported, this is in part due to the inclusion of Tylan 100®.

4. Conclusion

From the results of this study conducted at a commercial feedlot the following can be concluded. There is no impact of LFP supplementation at both 5 g/head/day and 10 g/head/day on body weight gain. All three treatments (LFP-5, LFP-10 and control) followed similar trends in body weight gain contradicting the reported 9% increase in body weight gain of LFP fed steers reported in U.S.A trials. There was also no observed improvement of ADG of the treatment groups versus the control. All three groups of steers performed equally through to day 107, upon which an atypical drop in ADG was observed until slaughter (at day 134). The DMI achieved in this experiment was lower than what would be generally expected in commercial feedlots due to various reasons, as discussed in this chapter. There were no differences in DMI between control and LFP treatments. An improvement of feed efficiency was reported in this experiment and the effect appears to be dose dependant. Steers supplemented with LFP-5 had a better feed conversion ratio when compared to the control and LFP-10 treatment, 4.82 and 4.86 vs 4.71 ($P<0.05$). This led to an overall FCR improvement of 2.5% for the

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LFP-5 fed steers over the control and LFP-10 treatment groups. This is, however, lower than the reported 6% improvement in feed efficiency in Texas, U.S.A. Consideration for future LFP trials needs to be made as to whether this improvement would be present under South African conditions if ADG was not restricted due to unexplained reasons.

Health data reported from this trial indicates that there was no advantage of feeding LFP on the health of feedlot cattle under typical South African conditions. The morbidities reported in this trial are slightly higher than what would be expected under commercial true commercial conditions. The suggested cause mentioned previously in this chapter deems it not to be a concern in the outcome of the experiment. Future research should take into account that numerically LFP-10 had the highest morbidity (approximately 13% higher when compared to the control). This could have possibly masked any effect LFP could have had on the above mentioned performance parameters.

There was no difference ($P>0.05$) in any of the measured carcass parameters. Hot and cold carcass weights were very similar and there was a consistent loss of approximately 1.5% across all three treatments from hot to cold carcass weights. There was no impact on dressing percentage as well as no effect on carcass classification. Very low rumen lesion scores reported in this study could have been due to the lower DMI from the reduced palatability of the feed, leading to smaller meal consumption and a less severe drop in rumen pH.

There is a vast need for future research on LFP supplementation in beef cattle. As with many natural feed additives inconsistencies in response, as in this study, are reported, consideration must be made for increased numbers of animals to better elicit the effect of feeding LFP on the health, performance and carcass characteristics of feedlot steers. It is also possible that the conditions in the rumen were not “unfavourable” enough.

It can be concluded that results from this trial suggest that LFP did not improve the overall performance of feedlot cattle fed a diet containing a mean inclusion rate of 25% roughage

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Chapter 4

Effect of a natural fermentation liquid supplement on rumen fermentation dynamics in beef feedlot cattle

1. Introduction

In order to address some of the aspects from the results of the commercial study described in Chapter 3 as well as underlying aspects from documents in the literature review, a research trial was designed to test and evaluate the fermentation dynamics and rumen pH effects of diets supplemented with LFP. The experimental design was a 3x3 Latin square design using the three finisher feedlot diets used in the commercial study in Chapter 3 as the experimental treatments. The aim of this experiment was to establish the potential mode of action of LFP liquid in the rumen and its influence on rumen fermentation parameters namely; volatile fatty acid production, rumen pH, *in sacco* DM, and NDF disappearance and rumen ammonia concentration.

2. Materials and methods

2.1. Experimental animals

Three, 6-7 year old Beefmaster –type, rumen cannulated steers were used in a 3 x 3 Latin square design to determine the effect of varying levels of the LFP liquid on rumen fermentation and digestibility. Upon arrival at the trial facilities the steers were steadily adapted from an *Eragrostis curvula* hay onto the finisher diet listed in table 3.1 for 21 days. Once the steers had been successfully adapted and consuming the finisher *ad libitum* they entered into the trial period. The rumen fermentation study was conducted for only the finisher diet as it contributed to the majority of the feeding period. With the following treatments: 0 gram/head/day (Control), 5 gram/head/day (LFP-5), 10 gram/head/day (LFP-10). LFP administration was done by spraying the LFP liquid onto the feed in the feed mixer during the mixing process. The animals were individually penned for the experimental period in a facility that simulated the commercial feedlot conditions. Body weight was recorded before feeding at the beginning of each period and after each period ended. Allocation of diets to the experimental animals is shown in Table 4.1.

Table 4.1 Allocation of experimental diets

	Animal Number		
Period	1	2	3
1	LFP - 5[1]	LFP - 10	Control
2	LFP - 10[1]	Control	LFP - 5
3	Control[1]	LFP - 5	LFP - 10

[1] Experimental Diets: Control (0 gram LFP per head per day); LFP - 5 (5 gram LFP per head per day); LFP - 10 (10gram LFP per head per day)

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2.2. Management

Animals were fed twice daily at 06:00 and 11:00. With bunk management being performed identical to the commercial application at Chalmar Beef to a) simulate commercial conditions and b) to replicate *ad libitum* feed intake whilst offering fresh feed twice a day. Clean water was available *ad libitum* for the duration of the trial. Orts were weighed back at the end of the sampling period to calculate actual DMI.

2.3. Experimental design

Each of the three periods within the Latin square comprised of 11 days, 7 days thereof for adaptation and the remaining 4 days for rumen sampling. The majority of Latin square designs used for testing feed additives, allows for 10-14 days of adaptation. However, due to the researcher's financial constraints a 7 day adaptation period was deemed viable and in agreement work done by other authors such as Sar *et al*. (2004), 7 days, and Castillejos *et al.*(2007) 6 to 14 days. Castillejos *et al.* (2007) reported that an adaptation period of at least 6 days is required to observe an effect on VFA concentrations and proportions. The sampling period was carried out by the collection of rumen samples 3 times daily, every 8 hours, progressing 2 hours each day from days 2 to 4. This sampling schedule provided 12 representative rumen fluid samples taken at 2 hour intervals. See Table 4.2 for feeding and sampling schedule. This sampling schedule has been used successfully by many researchers (Swinney-Floyd *et al.,* 1999b; Vermaak, 2011)

2.4. Sampling

Cattle remained in their designated pens for the entire feeding period, only being pulled for sampling, which was done in a standard cattle crush approximately 10 meters from the residing pens. The following rumen sampling procedure was followed: Pooled 60 ml samples of rumen fluid collected from 5 areas: anterior dorsal, anterior ventral, medium ventral, posterior ventral and posterior dorsal. These samples were collected using a 60 ml syringe with a 250 µm mesh at the collection point, supplied by Bar Diamond Inc. Parma, ID, USA. From each 60 ml sample filtrate, 30 ml was preserved with 5 ml of 50% H_2SO_4 and frozen at – 20^oC for NH_3-N analysis (Beauchemin *et al.,* 2003a). Twenty ml thereof was preserved with 4 ml 25% H_3PO_4 and frozen for volatile fatty acid analysis (Beauchemin *et al.,* 2003a) and 10 ml the remaining was frozen and stored for lactic acid analysis using a modification of the Barker-Summerson method as cited by Pryce (1969).

The samples were then pooled into 4 periods. Period 1 consisting of the first 6 hours post feeding (08:00,10:00, 12:00) period 2 the subsequent 6 hours period (14:00, 16:00, 18:00) and period 3 being the next 6 hours (20:00, 22:00, 24:00) and then finally period 4 the remaining 6 hours of the 24 hours representative day (02:00, 04:00, 06:00). Representative sampling and pooling was done using a magnetic stirrer after thawing at the UP Nutrilab just prior to sample preparation for the volatile fatty acid and lactic acid analyses.

All samples were analyzed at UP Nutrilab (University of Pretoria, Pretoria, Gauteng).

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Table 4.2 Feeding and sampling schedule of the experimental animals during the sampling periods

	Day 1	Day 2	Day 3	Day 4
Time				
06h00	Feeding	Feeding	Feeding	Feeding
08h00	1) Sampling of rumen fluid 2)Insertion of pH instument and 18 nylon *in sacco* bags[1]	1) Removal of 6 nylon *in sacco* bags[2]		
09h00				
10h00		1) Sampling of rumen fluid		
11h00	Feeding	Feeding	Feeding	Feeding
12h00			1) Sampling of rumen fluid	
13h00				
14h00				1) Sampling of rumen fluid
15h00				
16h00	1) Sampling of rumen fluid 2) Removal of 6 nylon *in sacco* bags[2]			
17h00				
18h00		1) Sampling of rumen fluid		
19h00				
20h00			1) Sampling of rumen fluid	
21h00				
22h00				1) Sampling of rumen fluid
23h00				
24h00	1) Sampling of rumen fluid 2) Removal of 6 nylon *in sacco* bags[2]			
01h00				
02h00		1) Sampling of rumen fluid		
03h00				
04h00			1) Sampling of rumen fluid	
05h00				
06h00				1) Sampling of rumen fluid 2) Removal of pH instrument

[1] 18 nylon *in sacco* bags [a) 9 nylon bags containing Finisher diet b) 9 nylon bags containing whole maize plant (chopped)]

[2] Removal of 6 nylon *in sacco* bags [a) 3 containing Finisher diet b) 3 containing whole maize plant (chopped)]

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2.5. Rumen pH measurements

Rumen pH measurements were carried out using a submersible pH meter and data logger as designed and supplied by Mr. H.J. Vermaak of Essential Nutrient Systems, Highveld ext, South Africa. The design and assembly is well described by the author (Vermaak, 2011). The pH meter and data logger was calibrated before each measurement period; this was done by validation for periods of 24 hours measuring pH readings of common standard solutions at pH 4 and pH 7 (UP Nutrilab, University of Pretoria, Pretoria, Gauteng) respectively. Calibration and validation was done at room temperature with a mean of 24.5 °C. Windows compatible software package Omnilog Data Management version 1.75 (Intech Instruments Ltd. Christchurch, New Zealand) was used to capture the logged data from the validation test. There was no detectable pH drift in the 24 hour validation period. Readings were set at 1 minute logging intervals and the pH was found to remain stable at the standard pH 4 and pH 7. The rumen pH measurements were set at 5 minute logging intervals over the 4 days of rumen sampling. Thereafter the submersible pH measuring instrument was removed, cleaned and the data was downloaded and captured by the Omnilog Software. See table 4.2 for insertion and removal schedule.

The rumen pH data was then pooled; mean pH taken for two hourly intervals during the entire sampling period. Total time spent above and below pH 5.5 was also calculated.

2.6. *In sacco* DM and NDF disappearance measurement

The determination of DM and NDF disappearance was done by an adapted *in sacco* method as described by Huntington and Givens (1995) which was based on initial work by Ørskov, and McDonald (1979). For each treatment and period within the 3x3 Latin square both the total mixed ration (finisher diet; Control, LFP-5 or LFP-10) and the diet roughage source (whole chopped maize plant) were accurately weighed (approximately 5 g) into twenty four (12 TMR and 12 roughage) nylon *in sacco* bags with a pore size of 42 µm (dimensions 50x120mm). Both roughage and TMR sources were ground in a Beaver lab 2682 mill (Asbestos Grading Equipment Co., Johannesburg, Gauteng, South Africa) through a 2-mm screen before weighing into the nylon bags for incubation in the rumen. Eighteen of the bags were evenly distributed in a durable nylon mesh sleeve with a weight at the bottom (to ensure the bags remain submersed in the rumen contents). This was repeated for each treatment (control, LFP-5, LFP-10). Upon commencement of the sampling period, 08:00 on day 1, the three sleeves measuring 600 mm in length were secured to the inside of the cannula stoppers of the experiment animals to facilitate removal and to inhibit the assembly from exiting the rumen.

After eight hours of being suspended in the rumen; six nylon bags (three TMR and three roughage) were removed from each of the experimental animals. The bags were then washed in a Sputnik portable washing machine (The Laundry Alternative, Johannesburg, South Africa) for 10min and frozen at -20 °C pending analysis. This was then repeated after another eight hours (sixteen hours post insertion) and again eight hours thereafter (twenty four hours after insertion). The remaining six (per treatment) zero hour bags (three TMR, three roughage) were washed and then frozen pending laboratory analysis. See Table 4.2 for *in sacco* sampling schedule and Table 4.3 for all the samples taken during the *in sacco* DM and NDF disappearance experiment.

All samples were dried, weighed and then removed from the nylon *in sacco* bags. Thereafter the samples were analysed for neutral detergent fiber (NDF) according to the method described by Van Soest and Robertson (1997). All NDF analyses were conducted in duplicate and were conducted at UP Nutrilab, University of Pretoria, Gauteng.

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Table 4.3 *In sacco* sampling procedure and sampling layout

	Period 1						Period 2						Period 3					
	Animal 1		Animal 2		Animal 3		Animal 1		Animal 2		Animal 3		Animal 1		Animal 2		Animal 3	
	No. of samples		No. of samples		No. of samples		No. of samples		No. of samples		No. of samples		No. of samples		No. of samples		No. of samples	
	LFP - 5	Roughage	LFP - 10	Roughage	Control	Roughage	LFP - 10	Roughage	Control	Roughage	LFP - 5	Roughage	Control	Roughage	LFP - 5	Roughage	LFP - 10	Roughage
Time (Hours)																		
0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
8	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
16	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
24	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Total	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**

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2.7. Statistical analysis

The data was analysed using the Proc GLM model (Statistical Analysis Systems, 2011) analysis which is suited for the mixed effects models in a 3x3 Latin Square Design. The model is shown below:

$$y_{ijk} = \mu + T_i + P_j + A_k + \varepsilon_{ijk}$$

Where y_{ijk} is the response due to each variable measure, μ is the overall mean, T_i is the treatment effects, P_j is the period effects, A_k is the animal effects and ε_{ijk} is the error. The animals were specified as random and the periods as repeated measurements. The covariances were identified as the compound symmetry assumption. Standard errors of the mean (SE^2) were calculated and significance was declared at $P<0.05$ and tendencies at $P<0.10$ using the Fisher test (Samuels, 1989).

3. Results and discussion

3.1. Effect of different levels of LFP supplementation on ruminal volatile fatty acid production

3.1.1. Total and individual volatile fatty acid concentrations

The total VFA's and proportions of the three major VFA's for this experiment are shown in Table 4.4. The average total VFA concentration over 24 hour period ranged between 98.6 mmol/L and 103.6 mmol/L. These values are comparable with the results achieved by other authors reporting on similar diets with high concentrate levels (Shain *et al.,* 1999). Acetate to propionate ratios reported in feedlot cattle studies range between 1.41 and 2.46 (Vermaak, 2011, Beauchemin *et al.,* 2003b). Acetate to propionate ratio's shown in Table 4.4 are reasonably low compared to the control diets in these studies suggesting the animals were possibly experiencing sub-acute acidosis (Khafipour *et al.,* 2009, Vermaak 2011). The low values reported in this study are comparable with acetate to propionate ratios reported by Khafipour *et al.* (2009), where authors reported an acetate to propionate ratio of 1.61 in dairy cows in the sub-acute rumen acidosis induced treatment group, this was due to the elevation of the propionate concentrations and reduction in acetate levels. In this study none of the animals displayed physical signs of sub-acute rumen acidosis such as reduction in feed intake or reported change in faecal appearance. Figure 4.1 illustrates the total VFA concentration over a period of twenty four hours.

There were no differences in any of the parameters shown in Table 4.4, suggesting there was no effect of LFP treatment on total or individual VFA production in the rumen ($P>0.05$).

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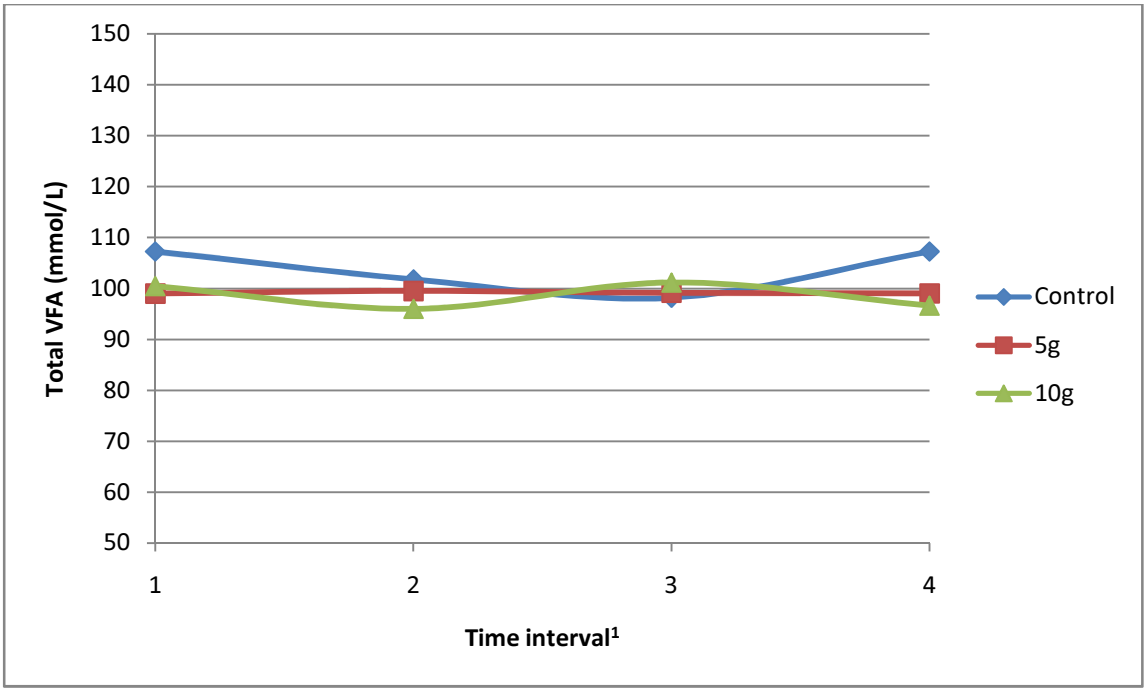
Table 4.4 Mean molar proportions and total volatile fatty acid concentrations produced by steers fed the treatments of LFP

	Treatment[1]			
	Control	5 g	10 g	± SE[2]
VFA concentration (mmol/l)				
Total	103.6	99.2	98.6	±0.587
Acetate	59.1	52.8	54.9	±0.893
Propionate	33.4	35.5	32.9	±0.489
Butyrate	8.89	8.17	8.59	±0.687
Individual VFA (molar %)				
Acetate	57.1	53.3	55.7	±0.986
Propionate	32.2	35.8	33.4	±0.477
Butyrate	8.58	8.24	8.72	±0.487
Acetate : Propionate Ratio	1.77	1.49	1.67	±0.469

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

[2] Standard error



[1] Time interval: **1** (08h00,10h00,12h00), **2** (14h000,16h00,18h00), **3** (20h00,22h00,24h00), **4** (02h00,04h00,06h00)

Figure 4.1 Effect of LFP supplementation on total volatile fatty acid concentrations produced in the rumen at different time periods post feeding

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3.1.2. Ruminal acetate concentrations

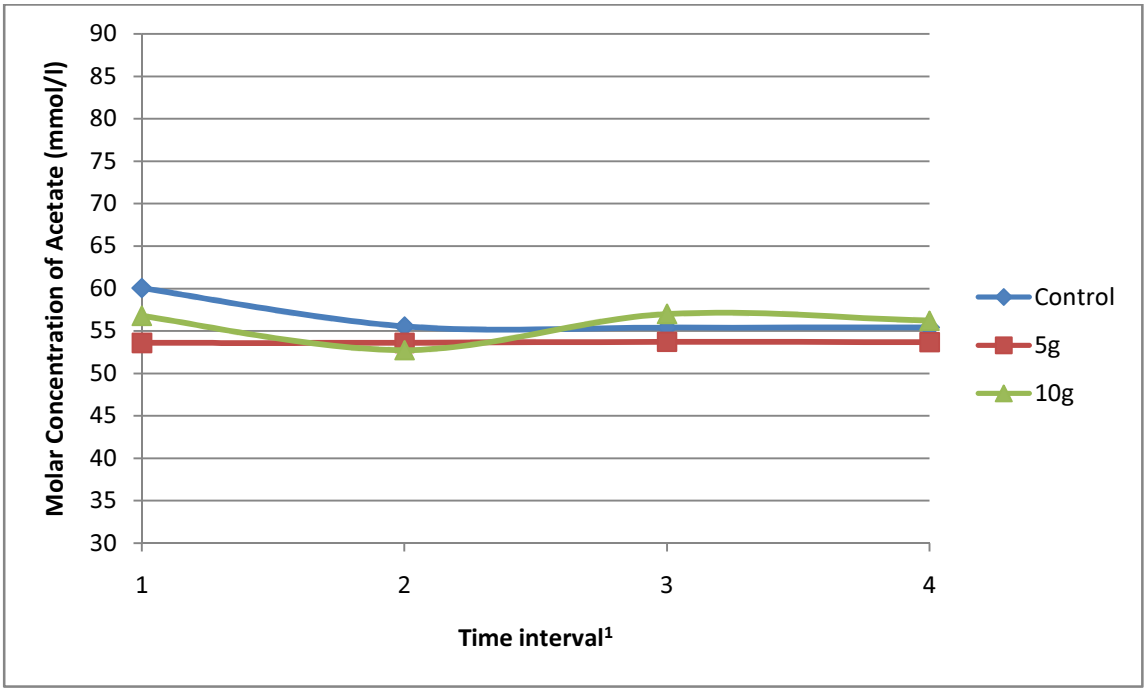
Table 4.5 represents the data obtained from the analysis for rumen acetate concentration sampled from the experimental animals. Molar acetate levels were lower than diets with similar concentrate levels such as those results found by Soita *et al.* (2003) and Vermaak (2011). The general depression of acetate is associated with low ruminal pH (Soita *et al.,* 2003, Vermaak 2011) which will be discussed later in this chapter. No significant effects ($P>0.05$) of LFP supplementation on ruminal acetate concentrations are indicated by these results.

Table 4.5 Molar concentrations of acetate over time produced by steers supplemented with different levels of LFP

	Treatments			
	Control	5g	10g	± SE[2]
Time interval[1]	mmol/l	mmol/l	mmol/l	
1	60.06	53.58	56.75	±3.168
2	55.53	53.59	52.73	±1.182
3	55.38	53.72	56.98	±1.310
4	55.39	53.69	56.21	±0.902

[ab] Row means with the same superscript do not differ significantly ($P>0.05$)
[1] Time intervals: **1** (08h00,10h00,12h00), **2** (14h000,16h00,18h00), **3** (20h00,22h00,24h00), **4** (02h00,04h00,06h00)

[2] Standard error



[1] Time intervals: **1** (08h00,10h00,12h00), **2** (14h000,16h00,18h00), **3** (20h00,22h00,24h00), **4** (02h00,04h00,06h00)

Figure 4.2 Molar concentrations of acetate over time produced by steers supplemented with different levels of LFP

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3.1.3. Ruminal propionate concentrations

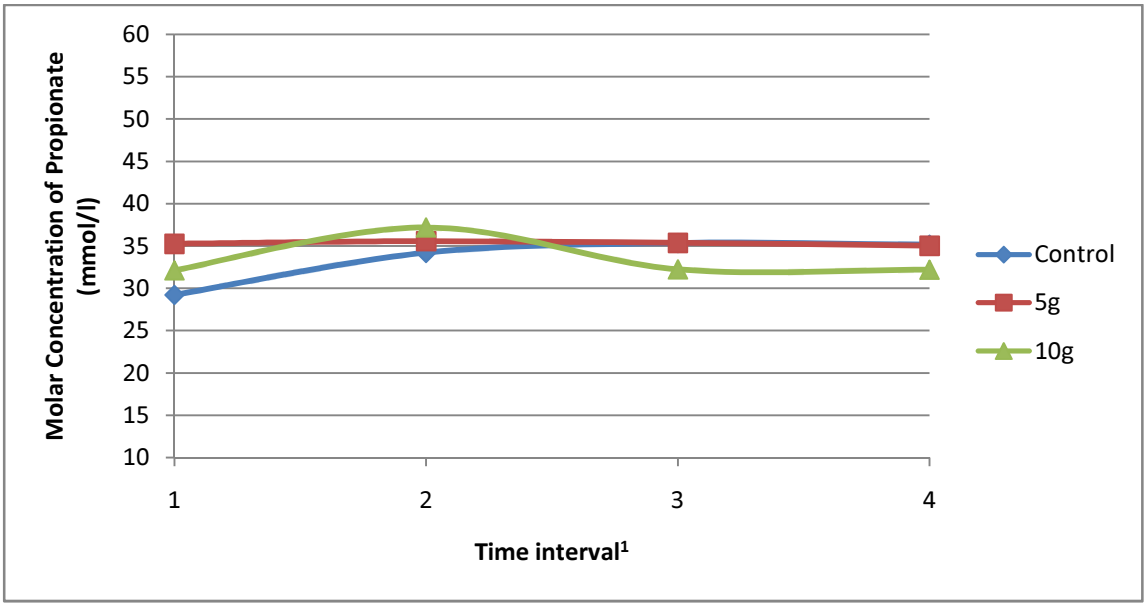
Table 4.6 represents the data obtained from the analysis for rumen propionate concentration sampled from the rumen cannulated steers. The ruminal propionate concentrations reported in this experiment are in agreement with concentrations from other feedlot studies (Vermaak, 2011). From Figure 4.3 it can be observed that LFP-5 treatment propionate production does not seem to be affected by feed intake. The data indicates that in the final period, period 4, post feeding control and LFP-10 treatments attained higher ruminal propionate than LFP-5 treatment, 35.16 and 35.02 vs 32.21 (P>0.05). It is concluded that in this experiment LFP supplementation had no effect (P>0.05) on ruminal propionate production.

Table 4.6 Molar concentrations of propionate over time produced by steers supplemented with different levels of LFP

	Treatments			
	Control	5 g	10 g	± SE[2]
Time interval[1]	mmol/l	mmol/l	mmol/l	
1	29.21	35.25	32.13	±3.095
2	34.19	35.56	37.18	±2.254
3	35.35	35.36	32.25	±0.959
4	35.16[a]	35.02[a]	32.21[b]	±0.205

[ab] Row means with the same superscript do not differ significantly (P>0.05)
[1] Time intervals: **1** (08h00,10h00,12h00), **2** (14h000,16h00,18h00), **3** (20h00,22h00,24h00), **4** (02h00,04h00,06h00)

[2] Standard error



[1] Time intervals: **1** (08h00,10h00,12h00), **2** (14h000,16h00,18h00), **3** (20h00,22h00,24h00), **4** (02h00,04h00,06h00)

Figure 4.3 Molar concentrations of propionate over time produced by steers supplemented with different levels of LFP

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3.1.4. Ruminal butyrate concentrations

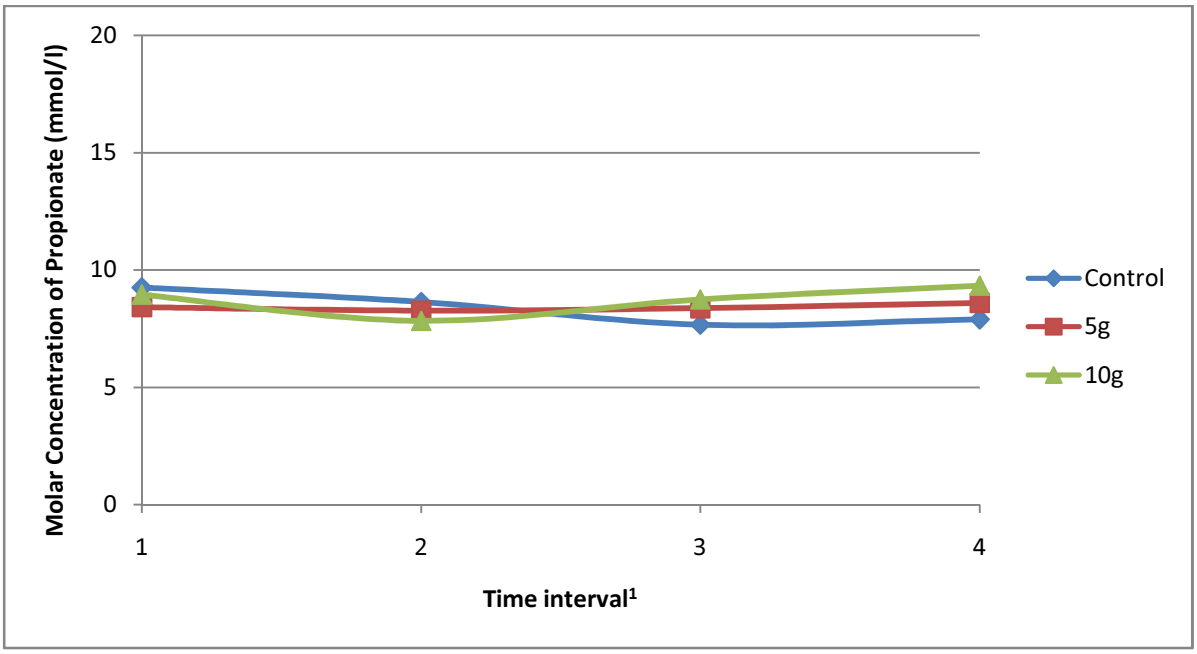
Rumen butyrate concentration collected from the cannulated steers in this experiment at different periods is shown in Table 4.7. The ruminal butyrate concentrations reported in this study are similar to those reported by other studies of feedlot diets (Vermaak, 2011). Vermaak (2011) reported a rumen butyrate concentration range of 6.03 mmol/l to 19.15 mmol/l. The range of rumen butyrate concentration reported in this study is, however, only 7.67 mmol/l to 9.33 mmol/l. This can in part be explained by the pooling of three of the two hourly samples as opposed to the single sample collected every four hours reported by Vermaak (2011). From Figure 4.4 it can be observed that LFP-5 treatments ruminal butyrate production does, once again, not seem to be affected by feed intake or at least a discernable intake pattern. It is concluded that in this experiment LFP supplementation had no effect (P>0.05) on ruminal butyrate production.

Table 4.7 Molar concentrations of butyrate over time produced by steers supplemented with different levels of LFP

	Treatments			
	Control	5 g	10 g	± SE[2]
Time interval[1]	mmol/l	mmol/l	mmol/l	
1	9.24	8.42	8.94	±0.350
2	8.63	8.26	7.83	±0.957
3	7.67	8.37	8.74	±0.496
4	7.89	8.59	9.33	±0.404

[1] Time intervals: **1** (08h00,10h00,12h00), **2** (14h000,16h00,18h00), **3** (20h00,22h00,24h00), **4** (02h00,04h00,06h00)

[2] Standard error



[1] Time intervals: **1** (08h00,10h00,12h00), **2** (14h000,16h00,18h00), **3** (20h00,22h00,24h00), **4** (02h00,04h00,06h00)

Figure 4.4 Molar concentrations of butyrate over time produced by steers supplemented with different levels of LFP

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3.2. Effect of different levels of LFP on ruminal ammonia nitrogen concentration

The effects of LFP source on rumen ammonia nitrogen (NH_3-N) concentrations at various time intervals are shown in Table 4.8 and illustrated in Figure 4.5. The mean NH_3-N concentrations in this experiment are in relative agreement with results found by Calsamiglia *et al.* (2002) and Rotger *et al.* (2005). The mean NH_3-N over the 24 hours for all treatments ranged between 8.40 and 11.40 mg NH_3-N /100 ml. These values were in general higher the suggested minimum levels of 5mg NH_3-N /100 ml required for optimal microbial protein synthesis (Satter and Slyter, 1975; Kennedy and Doyle, 1992). There was only one significant (P<0.05) difference between treatments this occurred at 22h00 (16 hours post feeding) between LFP-5 treatment and LFP-10 treatment (7.57 vs. 10.42). There was no difference (P>0.05) versus the control (8.80). This, however, is not biologically significant. It is concluded that in this experiment LFP supplementation had no effect (P>0.05) on rumen ammonia nitrogen production.

Table 4.8 Molar concentrations of NH3-N over time produced by steers supplemented with different levels of LFP

	Treatments			
	Control	5 g	10 g	± SE[2]
Time	mg/100 ml	mg/100 ml	mg/100 ml	
08h00	10.73	7.87	12.70	±0.898
10h00	11.90	10.61	12.88	±2.903
12h00	8.87	6.25	9.99	±2.521
14h00	8.59	8.16	9.19	±1.588
16h00	7.64	8.81	11.05	±1.426
18h00	10.27	9.96	13.70	±2.402
20h00	9.67	7.60	10.28	±1.843
22h00	8.80[ab]	7.57[a]	10.42[b]	±0.455
24h00	10.60	10.04	12.20	±2.587
02h00	10.43	8.00	11.86	±1.455
04h00	9.32	8.80	9.72	±1.838
06h00	9.24	7.11	10.15	±0.791

[ab] Row means with the same superscript do not differ significantly (P>0.05)

[2] Standard error

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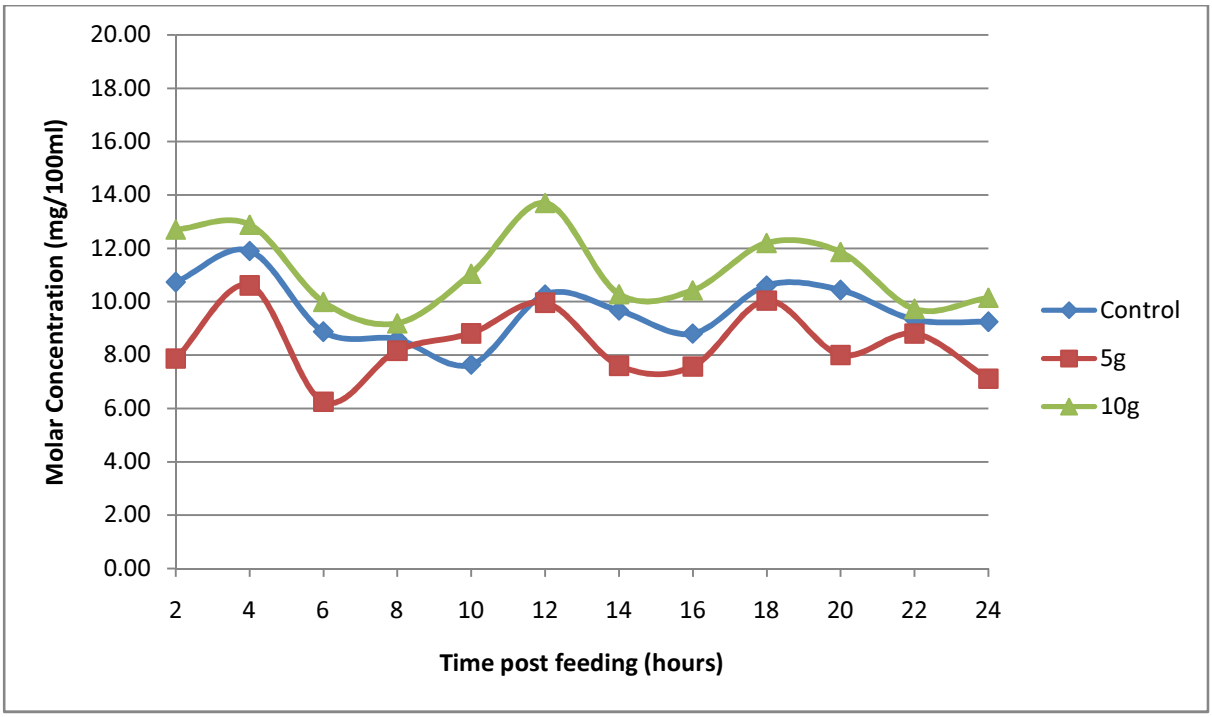

Figure 4.5 Molar concentrations of NH3-N over time produced by steers supplemented with different levels of LFP

3.3. Effect of different levels of LFP supplementation on ruminal lactic acid production

Table 4.9 and Figure 4.6 below show the molar concentrations of lactic acid over time periods by steers fed LFP-5, LFP-10 or control. The lactic acid concentrations recorded in this experiment are in agreement with results reported by other researchers (Burrin and Britton, 1986). There are, however, two anomalies in the data set from this study, lactic acid concentrations for the control and LFP-10 treatments during period four were extremely high. Khaipour *et al.* (2009) were able to induce sub-acute acidosis with lactic acid concentrations of 2.29 mmol/l, whereas the animals in this trial exhibited no reduced DMI at lactic acid levels reported to be more than double 2.29mmol/l. High lactic acid results which are comparable to these anomalies have been reported (Goad *et al.,* 1998). Authors reported lactic acid concentration of 4.9 mmol/l and 4.3 mmol/l in hay- and grain-adapted steers with induced sub-acute acidosis respectively. There were no differences between the different treatments in this experiment ($P>0.05$). The absence of statistical differences is probably due to the design of a 3x3 Latin square and the coefficient of variance being too large.

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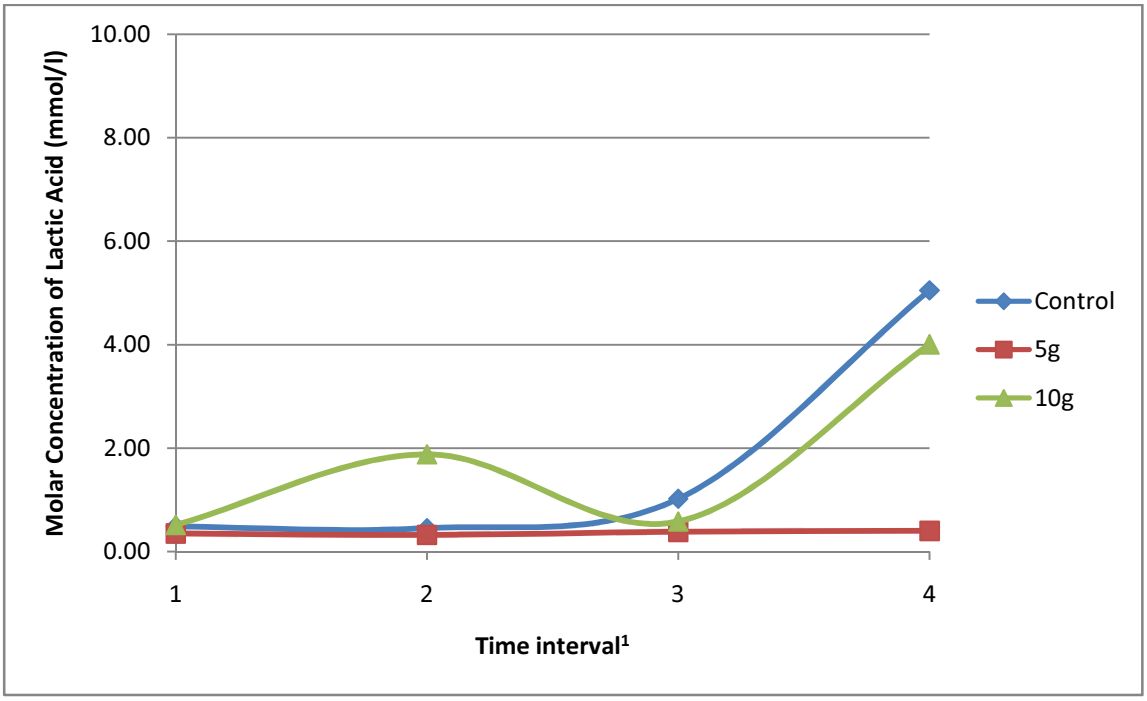
Table 4.9 Molar concentrations of lactic acid over time produced by steers supplemented with different levels of LFP

	Treatments			
	Control	5 g	10 g	± SE[2]
Time interval[1]	mmol/l	mmol/l	mmol/l	
1	0.48	0.35	0.51	±0.056
2	0.45	0.32	1.88	±0.803
3	1.02	0.38	0.58	±0.312
4	5.05	0.40	4.00	±3.480

[ab] Row means with the same superscript do not differ significantly (P>0.05)
[1] Time interval: **1** (08h00,10h00,12h00), **2** (14h000,16h00,18h00), **3** (20h00,22h00,24h00), **4** (02h00,04h00,06h00)

[2] Standard error



[1] Time intervals: **1** (08h00,10h00,12h00), **2** (14h000,16h00,18h00), **3** (20h00,22h00,24h00), **4** (02h00,04h00,06h00)

Figure 4.6 Molar concentrations of lactic acid over time produced by steers supplemented with different levels of LFP

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3.4. Effect of different levels of LFP supplementation on ruminal pH

The ruminal pH values recorded for the three different experimental diets are shown in Table 4.10 and illustrated in Figure 4.7. The rumen pH values in this experiment are in agreement with results reported by others (Moore *et al.,* 1987; Shain *et al.,* 1999; Beauchemin *et al.,* 2003b). Despite the ruminal pH values being in agreement, the rapid fluctuations are not as apparent as in other studies (Moore *et al.,* 1987; Shain *et al.,* 1999; Beauchemin *et al.,* 2003b). This can be explained by the fact that in the mentioned studies a single data point or pH reading was used at a specific time interval; whereas in this study the ruminal pH was recorded every 5 minutes and the data then pooled into 2 hourly intervals. Therefore, creating a mean value rather than a single pH reading, hence "masking" the recorded fluctuations in ruminal pH. There was only one difference ($P<0.05$) between the different LFP treatment groups. The difference was recorded at 08:00 (2 hours post feeding) pH 5.71 vs. 5.64 vs. 5.71 for the control, LFP-5 and LFP 10 treatments respectively. According to the ruminal pH data there is little or no effect of feeding LFP at both 5 g/head/day and 10 g/head/day on rumen pH values. It is, however, important to note that the pooled rumen data points as depicted in Table 4.10 below are in fact pooled data points from 5 minute intervals for the 2 hourly period and not the data points themselves. Therefore as illustrated in Figure 4.7 the rumen pH appears to have only declined to a minimum pH of 5.50 for the control group. The rumen pH in fact declined as low as pH 5.24 as can be seen in Table 4.11 which shows the minimum, maximum and mean rumen pH values for all the unpooled data points that were collected.

Table 4.11 and Figure 4.8 depict the minimum, maximum and mean pH for all the unpooled data points collected using the rumen pH probes and data loggers. The only difference in the unpooled 5 minute interval readings was in the maximum pH where treatment LFP-10 differed from the control ($P<0.05$). Maximum pH of the unpooled 5 minute interval readings are in agreement with other feedlot studies (Beauchemin *et al.,* 2003b; Vermaak, 2011). Beauchemin *et al.* (2003b) reported maximum ruminal pH of feedlot steers supplemented with live yeast and control steers (pH 6.21 vs. 6.40). The minimum and mean pH values did not differ ($P>0.05$). The range of the mean pH values (5.61 to 5.65) is in agreement with other feedlot studies (Beauchemin *et al.,* 2003b; Vermaak, 2011).

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Table 4.10 The effect of LFP supplementation on rumen pH at 2 hour intervals post feeding

	Treatment[1]				*P*-value
Time[2]	0	5	10	SEM	Treatment
02:00	5.71[b]	5.64[a]	5.71[b]	0.01	0.05
04:00	5.68	5.68	5.73	0.05	0.52
06:00	5.63	5.66	5.72	0.03	0.62
08:00	5.64	5.63	5.67	0.02	0.09
10:00	5.61	5.64	5.64	0.06	0.52
12:00	5.60	5.60	5.64	0.02	0.45
14:00	5.50	5.56	5.56	0.05	0.77
16:00	5.51	5.57	5.58	0.05	0.70
18:00	5.61	5.59	5.63	0.06	0.35
20:00	5.66	5.59	5.65	0.06	0.37
22:00	5.60	5.62	5.66	0.08	0.29
24:00	5.61	5.66	5.64	0.04	0.16

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

[2]Time: Hours post feeding, feeding occurred at 06:00 (0:00)

[ab] Row means with the same superscript do not differ significantly (P>0.05)

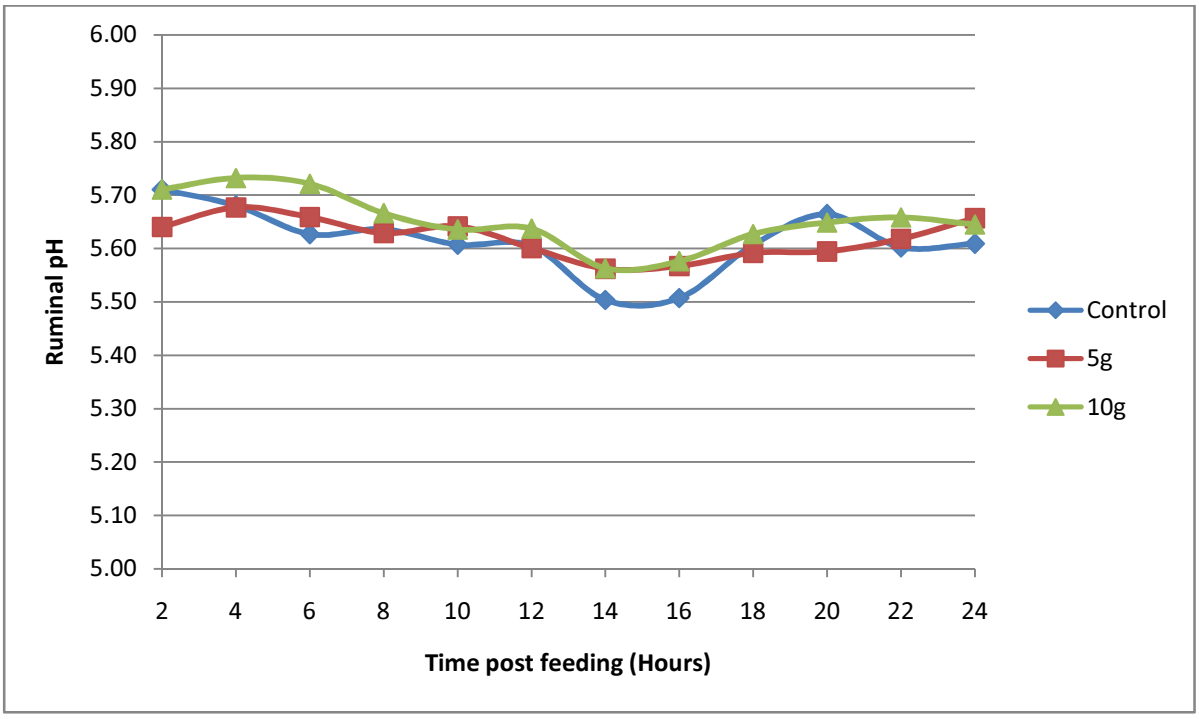

Figure 4.7 The effect of LFP supplementation on rumen pH at 2 hour intervals post feeding

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Table 4.11 The effect of LFP supplementation on rumen pH over a 96 hour period

	Treatment[1]				*P*-value
	0	5	10	SEM	Treatment
Rumen pH[2]					
Minimum pH	5.24	5.32	5.36	0.04	0.33
Maximum pH	6.13[a]	6.17[ab]	6.34[b]	0.08	0.03
Mean pH	5.61	5.62	5.65	0.04	0.66

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.
[2]pH: Minimum, Maximum and Mean pH recorded over 96 hour period

[ab] Row means with the same superscript do not differ significantly (P>0.05)

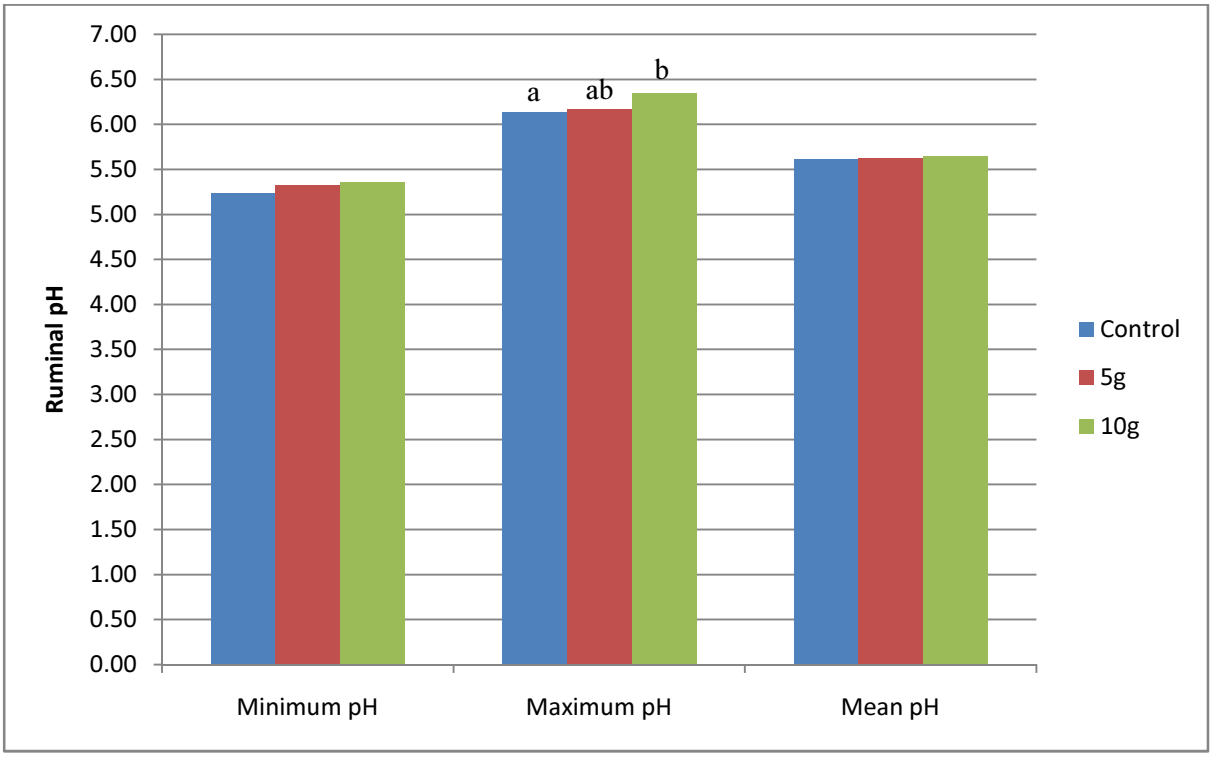

Figure 4.8 The effect of LFP supplementation on rumen pH over a 96 hour period

Sub-acute ruminal acidosis is generally characterised by ruminal pH between 5.6 and 5.2 (Owens *et al.,* 1998) and a ruminal pH below 5.2 is indicative of acute acidosis (Cooper and Klopfenstein, 1996). Sub-acute acidosis usually leads to feeding intake depression; however, even in metabolic studies researchers have difficulty consistently achieving sub-clinical responses (Cooper *et al.,* 1998). It is postulated by some authors (Owens *et al.,* 1998) that sub-acute and acute acidosis are rather a response to time spent below a pH threshold rather than the lowest pH point *per se.* The time spent, in minutes, below and above pH 5.5 is shown in Table 4.12 and illustrated in Figure 4.9.

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Despite the differences being vast numerically the degrees of freedom for experimental error of the 3x3 Latin Square did not lend itself to these being significantly different from one another (P>0.05) (Giri and Das, 1986). As in the minimum, maximum and mean ruminal pH parameters, the LFP-10 treatment spent the most minutes above pH 5.5 compared to the control and LFP-5 treatments. Naturally it is also the treatment that then spent the least amount of time below pH 5.5. The proportion, as a percentage, of time spent above and below pH 5.5 is shown in Table 4.12. The proportion of time spent below pH 5.5 is greater than other studies on feedlot steers consuming high concentrate diets (Beauchemin *et al.,* 2003b). Authors reported steers spent 50.1% and 59.5% below pH 5.5 for control and yeast supplemented steers respectively. In future studies of this kind it is advised that a replicated 3x3 Latin Square design should be used.

Table 4.12 The effect of LFP supplementation on the time spent above or below pH 5.5 during a 96 hour period

	Treatment[1]				*P*-value
	0	5	10	SEM	Treatment
Time[2]					
Minutes ≤ pH 5.5	1975.33	1596.67	1358.33	631.94	0.67
Minutes ≥ pH 5.5	3731.67	4140.00	4346.67	615.43	0.65
% Time[3]					
% ≤ pH 5.5	34.6	27.8	23.8	11.06	0.55
% ≥ pH 5.5	65.4	72.2	76.2	10.77	0.50

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.
[2]Time: Cumulative time spent above or below pH 5.5 in minutes
[3]%Time: Proportion of time spent above or below pH 5.5

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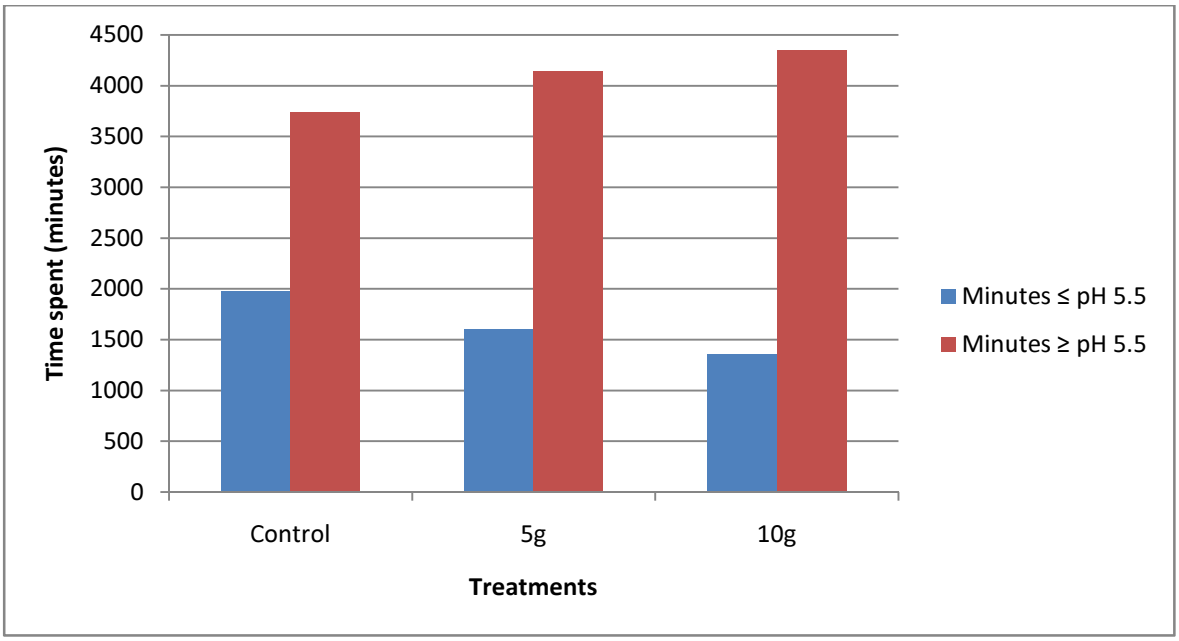

Figure 4.9 The effect of LFP supplementation on the time spent above or below pH 5.5 during a 96 hour period

3.5. Effect of different levels of LFP supplementation on ruminal NDF disappearance

3.5.1. Total Mixed Ration

The effect of LFP treatment on the *in sacco* NDF disappearance of the TMR are shown in Table 4.13. The results of this study are slightly higher on average than NDF disappearance data reported for TMR diets on dairy cows (Schroeder *et al.,* 2003). Authors reported NDF disappearance of 6.2% and 11.3% at 9- and 16 hours in the rumen, as opposed to this study mean 8.4% at 8 hours in the rumen and 23.2% at 16 hours. The NDF disappearance reported, at 24 hours, in this study are comparable with NDF disappearance of immature whole plant maize silage (Bal *et al.,* 2000). The disappearance of NDF, as a percentage, is illustrated in Figure 4.10a and Figure 4.10b.

The results of this study suggests that there is no effect of LFP supplementation on the ruminal NDF disappearance of the TMR (P>0.05).

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Table 4.13 The effect of LFP supplementation on *in sacco* NDF disappearance of the TMR (total mixed ration)

	Treatment[1]				*P*-value
	0	5	10	SEM	Treatment
Time[2]	%NDF[3]	%NDF	%NDF		
00:00	3.40	3.11	3.26	1.36	0.54
08:00	10.64	7.52	7.04	2.25	0.85
16:00	20.79	20.02	28.73	2.87	0.47
24:00	31.02	31.82	38.34	2.07	0.22

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

[2]Time: Hours after insertion of the dacron bags, insertion occured at 06:00 (0:00)

[3]NDF disappearance (%DM)

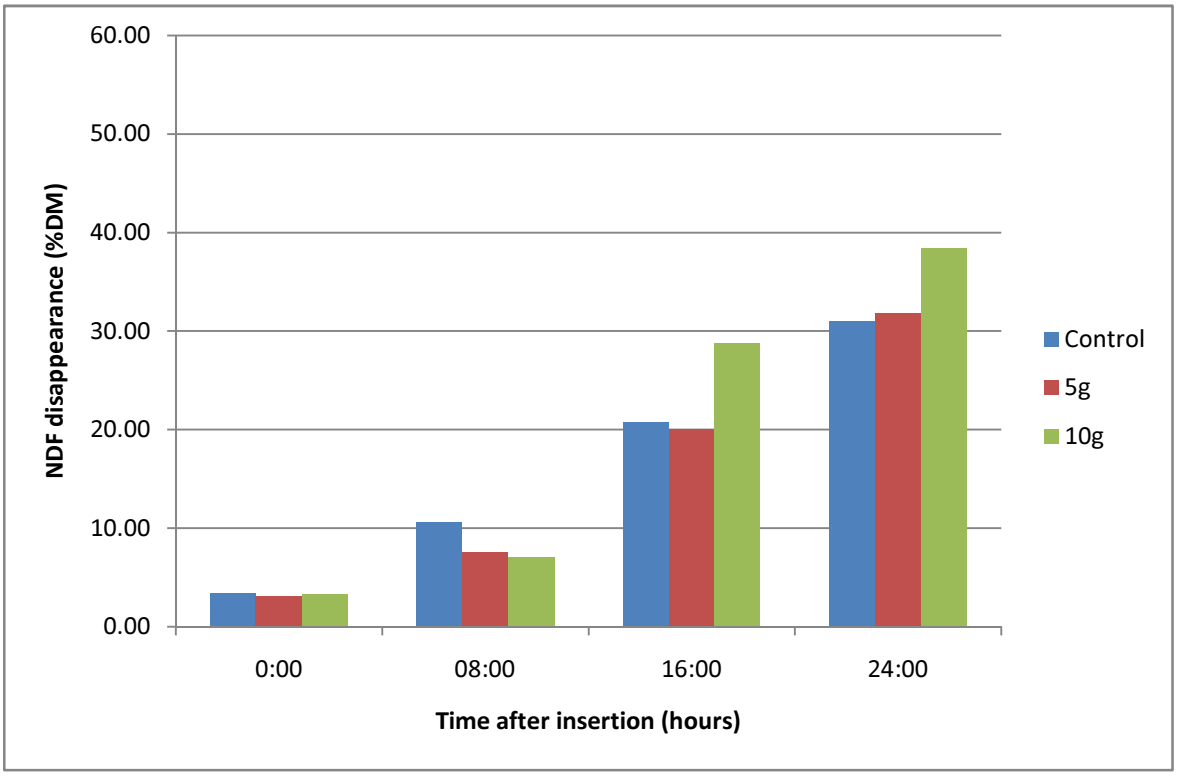

Figure 4.10a The effect of LFP supplementation on *in sacco* NDF disappearance of the TMR (total mixed ration)

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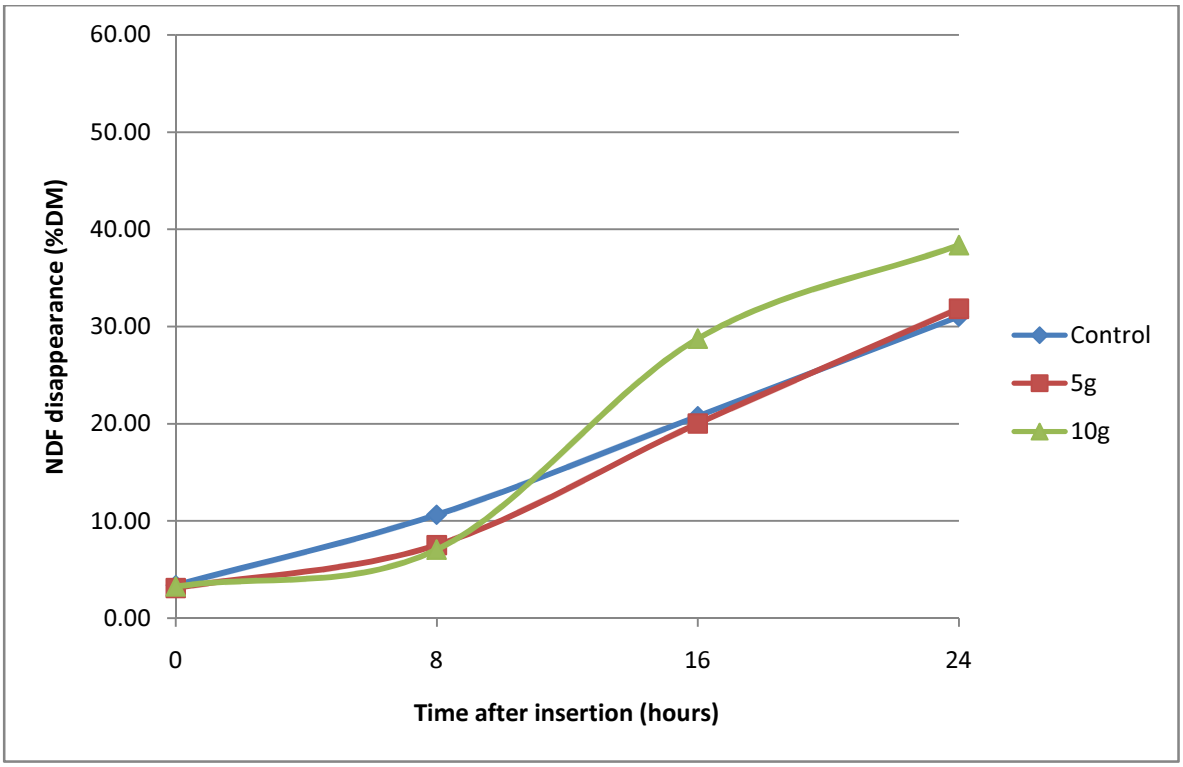

Figure 4.10b The effect of LFP supplementation on *in sacco* NDF disappearance of the TMR (total mixed ration)

3.5.2. Roughage Source

The effect of LFP treatment on the *in sacco* NDF disappearance of the roughage source samples (whole chopped maize plant) are shown in Table 4.14. The results from this experiment are comparable to other experiments using low quality roughages in cattle (Chan, 1992; Bowman and Firkins, 1993; Koike *et al.,* 2003). The NDF disappearance was slightly higher (23.48% versus 14.5%) at 24 hours than that of Koike *et al.* (2003), this is due to the fact that authors used low quality orchard grass hay stems. The disappearance of NDF, as a percentage, is illustrated in Figure 4.11a and Figure 4.11b.

The results of this experiment suggest there is no effect (P>0.05) of LFP supplementation on NDF disappearance of the roughage source in this diet.

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Table 4.14 The effect of LFP supplementation on *in sacco* NDF disappearance of the diet roughage

	Treatment[1]				*P*-value
	0	5	10	SEM	Treatment
Time[2]	%NDF[3]	%NDF	%NDF		
00:00	2.52	2.36	2.72	0.75	0.27
08:00	12.47	10.89	12.15	0.49	0.11
16:00	21.19	22.29	19.69	0.96	0.47
24:00	23.94	24.38	22.13	0.96	0.39

[1]Treatment: 0 g *Lactobacillus* fermentation prototype per head per day; 5 g Lactobacillus fermentation prototype per head per day; 10 g Lactobacillus fermentation prototype per head per day; Diamond V, Cedar Rapids, Iowa, USA.

[2]Time: Hours after insertion of the dacron bags, insertion occured at 06:00 (0:00)

[3]NDF disappearance (%DM)

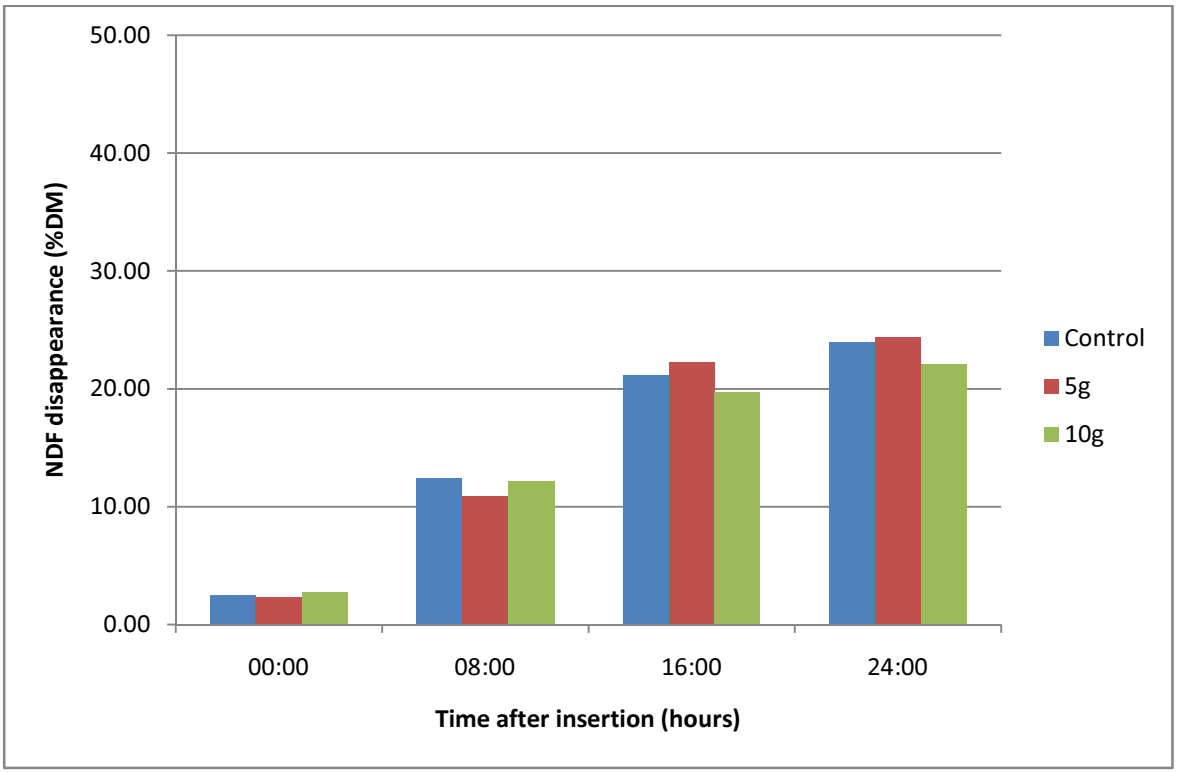

Figure 4.11a The effect of LFP supplementation on *in sacco* NDF disappearance of the diet roughage

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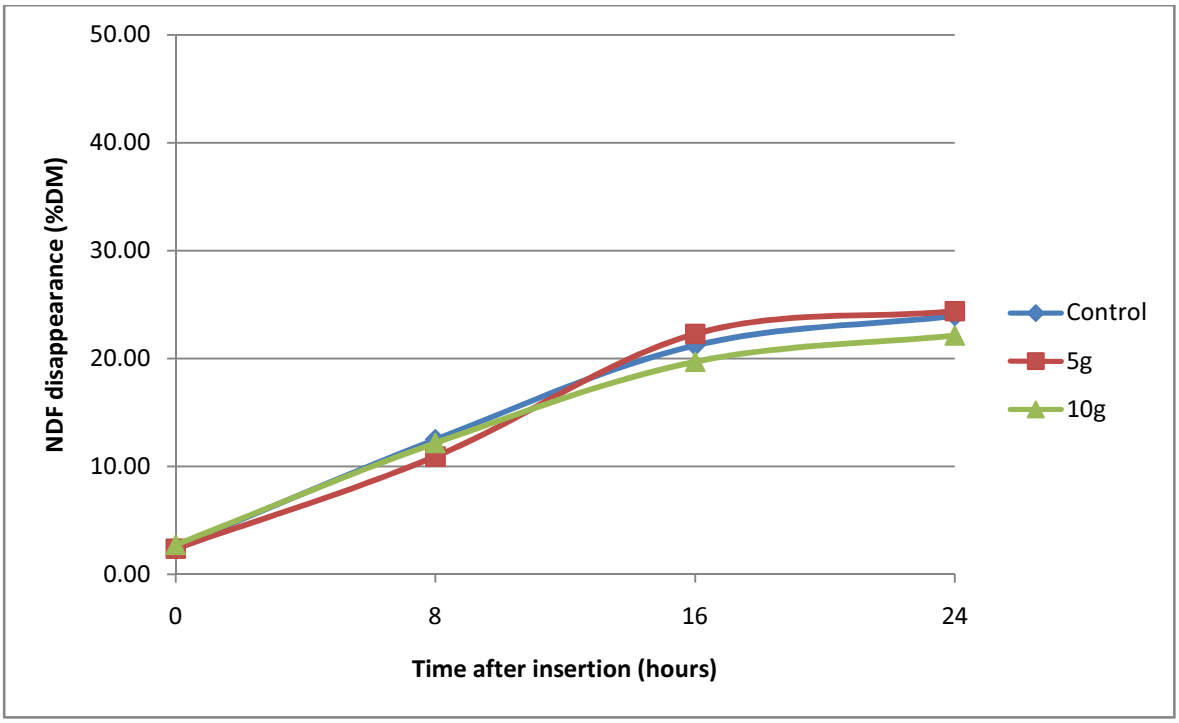

Figure 4.11b The effect of LFP supplementation on *in sacco* NDF disappearance of the diet roughage

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4. Conclusion

The results from this experiment suggest that LFP supplementation does not affect the total VFA production (P>0.05), and neither does it affect the molar proportions of the three major volatile fatty acids (acetate, propionate and butyrate) (P>0.05). Acetate to propionate ratio was also not affected by LFP supplement (P>0.05). The lactic acid production reported in this experiment were generally similar to concentrations shown by other authors feeding similar concentrate diets. The exception, however, is period four for control and LFP-10 where the lactic acid levels were relatively high but not significantly different (P>0.05) to LFP-5 treatment. It can, therefore, be concluded that LFP supplementation did not appear to have an effect on ruminal lactate production (P>0.05). Ruminal pH parameters were also not affected by LFP supplementation (P>0.05), which can be expected as ruminal pH is greatly influenced by VFA and lactic acid production. It can therefore be concluded that LFP supplementation has no effect on the energy fermentation dynamics in the rumen.

There was only one data point which indicated a difference (P<0.05) in ruminal NH_3-N production for the duration of the sampling period this, however, is not biologically significant. The conclusion, is therefore, that LFP has no effect on ruminal NH_3-N production (P>0.05).

NDF disappearance, *in sacco,* of both the total mixed ration and the roughage only, indicated no effect (P>0.05) of LFP supplementation on rumen NDF degradation.

Due to the restraints of the small size of the 3x3 Latin Square, there are very few degrees of freedom for experimental error (Giri and Das, 1986); further research into the mode of action of LFP on rumen fermentation parameters needs to be conducted using a larger experimental design e.g. 5x5 Latin Square or a replicated or triplicated 3x3 Latin square. The concluded results of this trial, however, are that LFP supplementation does not affect rumen fermentation dynamics in beef feedlot cattle.

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Chapter 5

General Discussion and Conclusion

This study was conducted to evaluate the efficacy of an all-natural liquid fermentation prototype (LFP) in South African feedlots diets. The objective of this study was to investigate the effects of the LFP at different doses on production parameters and to establish a possible mode of action. The steers sourced for the commercial study were very homogenous and an ideal representation of the type of cattle being fed in the majority of the feedlots in South Africa. The diets were formulated to be representative of a "common" diet being fed in South Africa. The exception was that of the whole chopped maize plant which is not commonly added at such high inclusion levels. The majority of feedlots use maize silage as a roughage source in the feedlot diets, in this case , however, due to the protocol of the mixing and feeding of the feed; omission of the maize silage was unavoidable to prevent feed from being spoilt. This led to a slightly lower feed intake than expected and lower than generally recorded under South African conditions, this fortunately occurred across the treatment groups and did not influence the data collected between treatments. Body weight gain and average daily gain were acceptable for representative South African feedlots. LFP supplementation did not appear to have any effect on gain, the variance of final body weights was 3 kg and that of ADG was 30 grams/head/day, this would generally be considered uniform across 142 cattle. LFP supplementation did, however, show an improvement on feed efficiency with an improvement on FCR of 2.5%. Contrary to what would be expected, the data from this trial suggests that the effect of LFP is dose dependent and in favor of 5 grams/head/day ($P<0.05$). The uniformity across treatments was also observed at slaughter on day 134 of the trial and no improvement from LFP substitution was observed. Carcass mass, dressing percentage and carcass classification was representative of South African steers not being fed beta-agonists in the finisher diets. No effect of LFP supplementation could be measured in the slaughtering data. As indicated in the literature review of this study many dietary factors influence the efficacy of DFM's or microbial cultures, therefore responses to LFP may vary significantly from diet to diet. It is postulated that future trials on silage based diets with higher dry matter and starch intake may yield results similar to those reported in the U.S.A (9% improvement on body weight gain and 6% improvement on FCR). Future research would also need to access LFP supplementation in the absence of ionophores and AGPs as they may also have an impact on the efficacy of the product in the rumen.

The rumen fermentation study that was conducted was to evaluate the influence of LFP supplementation on rumen fermentation dynamics and possibly establish a mode of action. Unfortunately the data from the trial was impaired by the fact that only 3 cannulated steers were available for the study. A 3 x 3 Latin square is subject to 2 degrees of freedom for experimental error, which in essence means that the effect of the treatment would need to be substantial and repeatable for it to be statistically significant. Substantial and repeatable results are not common (as indicated in chapter 2) amongst the majority of natural or alternative feed additives. There appears, from the literature review, to be too many dietary and ruminal interactions to overcome animal and period influences. Pooling of collected samples were done due to cost implications, which possibly masked the true variation occurring post-feeding. This could also possibly explain the lack of response in the LFP-5 treatment. This in addition to the limiting factors of the 3 x 3 Latin Square yielded no significant results between the control and the LFP treatments. In order to achieve plausible results changes to the experimental design would be required to attain the objectives set out for this study. At least 7 or 8 animals and limited sample pooling would need to be considered to achieve valuable data for the nature of these alternative feed additives.

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In conclusion, results from this study suggests LFP supplementation might have potential to affect beef feedlot performance, in particular FCR. Overall, however, none of the parameters such as BW gain, DMI, ADG, health status or carcass quality were affected by LFP supplementation. The rumen study, a 3 x 3 Latin square design with limited statistical power, failed to provide meaningful information regarding mode of action of the LFP supplement.

Implications

These trials were conducted in the presence of monensin and tylosin (only in the grower and finisher diets) and therefore not intended as a possible replacement for monensin and/or tylosin. The aim, therefore, was more focused on a possible additive or complimentary effect of LFP with monesin and tylosin. This was in part due to the fact that ionophores and AGPs are currently still permitted for use in South Africa and can be procured relatively inexpensively. Unless consumer pressure results in the withdrawal of ionophores and AGPs from feedlot diets, South African feedlot operators will continue to use efficiency enhancing products such as ionophores and AGP's to sustain profitability. Caution should be taken when considering the inclusion of feed additives into feedlot diets. Improvements in feed efficiency, such as these shown from the supplementation of LFP, do warrant the attention of feedlot operators and nutritionists. Feed efficiency, however, should not be the parameter by which the inclusion or omission of feed additives is judged as it does not reflect any economic benefit or lack thereof to the feedlot. Economic studies conducted in combination with performance experiments yield return on investment or gain over feed cost parameters which are superior tools for comparison of both antimicrobial and alternative or natural feed additives. An economic evaluation could not be performed since the product is only a prototype and not a commercially available additive. Based on the lack of response on the majority of parameters measured, it is doubtful whether the supplement would be economically viable.

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Chapter 6

Appendices

Table 6.1 Sensitivity of ruminal bacteria to ionophores (Adapted from Hobson & Stewart, 1997)

Bateria	Fermentation Products	Gram Strain	Cell Wall Type[2]	Sensitivity to Ionophores
Eubacterium spp.	C4[1]	+	+	Susceptible
Streptococcus bovis	Lactate, Form, C2	+	+	Susceptible
Lactobacillus spp.	Lactate	+	+	Susceptible
Clostridium spp.	NH_3	+	?	Susceptible
Peptostreptococcus anaerobis	NH_3	+	+	Susceptible
Ruminococcus	C2[1], H_2, Form	-	+	Susceptible
Butyrivibrio fibrosolvens	C2, C4, Form	-	+	Susceptible
Megashaera elsdenii	C3[1], Succinate	-	-	Resistant
Prevotella ruminicola	C3, Succinate	-	-	Resistant
Selenomonas ruminantium	C3, Succinate	-	-	Resistant
Methanobacterium spp.	C2, CH_4			Resistant
Methansarcina	CH_4			Resistant

[1] C2= Acetate; C3= Propionate ;C4= Butyrate
[2] Cell wall type + = Susceptible, - = Resistant

Table 6.2 Effects of sodium monensin treatment on the composition of ruminal fermentation products (Dinius *et al.*, 1976)

	Control	Sodium Monensin
Acetate (%)	66.70	61.30
Propionate (%)	20.10	26.10
Acetate : Propionate	3.3	2.40
Butyrate (%)	9.20	9.40
Total VFA, mM	77.80	74.90
Methane production, Moles/100 moles hexose	62.30	54.20

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