Effect of dietary energy and fibre source on rumen function in feedlot steers.

Henning Johannes Vermaak

Submitted in partial fulfilment of the requirements for the degree

MSc. (Agric) Nutrition Science (Animal Science)

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DECLARATION

I, Henning Johannes Vermaak declare that the dissertation, which I hereby submit for the degree MSc (Agric) Nutrition Science (Animal Science) 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.

SIGNATURE:.....

DATE: 18 July 2011

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Summary

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Co-supervisor: Prof L.J. Erasmus

Within the South African feedlot industry, there are currently several different roughage sources available for use in feedlot finishing diets. To evaluate the influence of dietary energy and fibre source on rumen function in feedlot animals, four roughage sources (wheat straw, *Eragrostis curvula* hay, cottonseed hulls and maize silage) were used in combination with hominy chop or dry rolled maize in two experiments. Four Beefmaster steers (270kg ±15kg) fitted with ruminal cannulae were used in two separate experiments in a 4 x 4 Latin square design. Experiment 1 was conducted to evaluate each roughage source in combination with hominy chop namely: wheat straw, hominy chop (**WSHC**); Eragrostis hay, hominy chop (**EHHC**); cottonseed hulls, hominy chop (**CHHC**); and maize silage, hominy chop (**MSHC**). Experiment 2 was conducted to evaluate each roughage source in combination with dry rolled maize (**DRM**) namely: wheat straw, dry rolled maize (**WSDRM**); *Eragrostis curvula* hay, dry rolled maize (**EHDRM**); cottonseed hulls, dry rolled maize (**CHDRM**); and maize silage, dry rolled maize (**MSDRM**). Diets were designed to contain equal amounts of energy, starch, crude protein, neutral detergent fibre (NDF) and 7.5% roughage source in both experiments. All diets were evaluated for particle size distribution through the Penn State Forage Particle Separator (**PSPS**) and ruminal fermentation parameters (volatile fatty acid composition, **VFA**; rumen ammonia nitrogen, **NH_3-N**; lactate and ruminal pH) were compared for each experiment. Results from experiment 1 showed that animals fed the MSHC had the lowest ($P<0.05$) concentration of VFA while animals fed the CHHC diet produced the highest ($P<0.05$) ruminal propionate concentration, lowest ($P<0.05$) acetate: propionate

ratio and had the lowest ($P<0.05$) ruminal pH during the 24h observation period. Time intervals below pH 5.6 and pH5.2 for CHHC was 940 minutes ($P<0.05$) and 388.75 minutes respectively. Measurements for rumen NH_3-N concentrations and lactate did not differ between treatments. Results from experiment 2 revealed that animals fed WSDRM had numerically the lowest concentration of VFA and differed ($P<0.05$) from CHDRM and MSDRM diets. Propionate and acetate as well as A:P ratios for CHDRM were numerically higher than other treatments but differed ($P<0.05$) from the WSDRM diet. Rumen NH_3-N concentrations did not differ but lactate concentrations were higher for EHDRM when compared to the MSDRM and WSDRM diets ($P>0.05$). Ruminal pH observations showed steers consuming the MSDRM diet to have the lowest mean ruminal pH of 5.53 which differed ($P<0.05$) from the WSDRM diet with a mean ruminal pH of 6.1. Time periods spent below pH 5.6 and 5.2 for steers consuming the MSDRM diet was highest at 703.75 and 306 minutes respectively and differed from steers consuming the WSDRM diet. Results from these experiments indicated that different roughage sources in combination with specific energy sources resulted in different rumen fermentation characteristics. Evaluation of particle size distribution from the roughage source, particularly the large pool (upper and middle sieve sizes on PSPS) further revealed that particle size alone does not explain all variation in fermentation patterns alone but the digestible NDF as percentage of total NDF for these fractions could be a valuable predictor for chewing and rumination activity to ultimately establish a more optimal ruminal pH.

LIST OF ABBREVATIONS

A: P	Acetate to propionate ratio
ADF	Acid detergent fibre
CEC	Cation exchange capacity
CF	Crude fibre
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
DIP	Degradable intake protein
DM	Dry matter
DMI	Dry matter intake
dNDF	Digestible neutral detergent fibre
EE	Ether extract
eNDF	Effective neutral detergent fibre
FC	Fibre carbohydrates
MCP	Microbial crude protein
ME	Metabolizable energy
MIU	Million international units
MJ	Mega Joule
Mcal	Mega calories
N	Nitrogen
NAN	Non ammonia nitrogen
NDF	Neutral detergent fibre
NEg	Net energy for gain
NE_L	Net energy for lactation
NEm	Net energy for maintenance
NFC	Non fiber carbohydrate
NFFS	Non-forage fiber source
NPN	Non protein nitrogen
NRC	National Research Council
NSC	Non-structural carbohydrate
PEF	Physical Effective Factor
peNDF	Physically effective neutral detergent fibre
PSPS	Penn State Forage Particle Separator
pK	pH point of maximum buffering
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
SEM	Standard error of the mean
TMR	Total mixed ration

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CHAPTER 1.

1.1 INTRODUCTION & MOTIVATION

Within the South African feedlot industry, there are currently several different roughage sources available for use in feedlot finishing diets. These roughage sources include maize silage, *Eragrostis curvula* hay, wheat straw, bagasse and cotton seed hulls. These roughage sources are normally fed as a sole source of roughage or in different combinations. Several sources of energy are used in combination with these roughage sources. These energy sources are predominantly dry rolled maize, whole maize and hominy chop. Hominy chop is a by-product of the South African maize milling industry. A commonality found within most hominy chop sources is the inconsistency in physical and chemical composition. The largest variation is found within the amount of fermentable carbohydrates. Variation in roughage or functional fibre sources is found primarily in particle size and in chemical composition. A majority of physical variation in particle size is created through different roughage processing methods as well as mixing processes at the feedlot.

Secondary to the problematic situation of attempting to feed high levels of energy with low roughage levels is the associated negative effects on ruminal fermentation. The most detrimental digestive influence on animal performance is sub-clinical ruminal acidosis. Factors such as variation in feed intake also influence rumen fermentation (Cooper *et al.,* 1999). Roughage particles rate of hydration may influence particle specific gravity followed by altering rumen reticulum retention time of forage particles and kinetics of concentrate particles (Wattiaux *et al*., 1992).

The relationship between roughage concentration in the diet and its effects on rumen fermentation and the resulting effects on cattle production is a well recognized field of research in ruminant nutrition. Roughage in feedlot diets is utilized as a source of functional fibre that elicits cattle chewing, stimulating saliva production (i.e. salivary glands). Saliva contains high levels of bicarbonate and quantities produced are directly correlated related to rumen buffering capacity or pH. The quantity of roughage within a ration stimulates feed intake as well as maintaining digestive health to maximize net energy for gain (NEg) intake by cattle (Defoor *et al*., 2002). Cost and roughage availability normally dictates the choice of roughage, but nutritionist's must consider how well a given roughage source complements a selected concentrate and in addition to source also consider the processing method (Owens *et al.,* 1997).

The purpose of this study was to evaluate the effect of different roughage sources and inclusion levels in combination with different energy sources, on rumen fermentation characteristics in typical South African feedlot diets. This will encompass differences found for roughage particle size, distribution within total mixed ration (TMR), effective neutral detergent fibre (eNDF) and physical effective neutral detergent

fibre (peNDF). The Penn State Forage Particle Separator (PSPS) has become widely accepted as a quick and practical method for routine use on the farm to evaluate particle size of roughages and TMR's (Lammers *et al.,* 1996). These observed measurements will differentiate between the experimental diets and address the concepts of physical effectiveness of fibre in the diets. These observations include measurements of diet, particle size distribution, ruminal pH, volatile fatty acid (VFA) composition and microbial protein production.

The emphasis of this study is to evaluate four different commonly used roughage sources in combination with two commonly used energy sources generally used in feedlot finisher diets. During two separate experiments these feedstuff combinations will be characterised and evaluated with regards to its effects on rumen fermentation patterns and ruminal pH fluctuations. The following chapter will present the various aspects with regards to effective fibre, neutral detergent fibre (NDF), source, rumen fermentation, particle size distribution and ruminal acidosis.

CHAPTER 2

2. LITERATURE REVIEW

2.1 INTRODUCTION

Feedlot cattle are commonly finished on high-concentrate diets to maximise growth efficiency and minimise cost of gain. Ruminants have evolved on grasslands and have in the process developed a digestive compartment called the reticulo-rumen which allows them to effectively make use of fibrous feedstuffs. These animals where never intend to be fed grains and haven't had access to grains for thousands of years. With westernisation grains became abundant to the extent where it could also be fed to ruminants. The cost per unit energy from grains is always cheaper than the cost per unit energy from fibrous feedstuffs, which in today's "high priced energy" world makes feeding grains to ruminants a very lucrative practice. Digestive disorders such as ruminal acidosis and bloat however, are commonly observed in feedlots today and our understanding and ability to diagnose these disorders is often hampered by the low frequency of occurrence. Well known nutritionist Rod Preston stated at the 1996 Midwest nutritional symposium that "Roughage is a nebulous term that should be discontinued, at least as it applies to feedlot cattle diets" According to him and other well known researchers in this field, effective neutral detergent fibre (eNDF) is probably a viable replacement concept since it far better predicts reticulo-rumen pH, especially when diets have less than 26% NDF.

2.2 DEFINING ROUGHAGE

The most common measures of fibre used for routine feed analysis are: Crude fibre, acid detergent fibre (ADF), and neutral detergent fibre (NDF). All these analysis, however, are problematic in the sense that none of these fractions are chemically uniform. Neutral detergent fibre measures most of the structural components in plant cells (cellulose, hemicellulose, and lignin). Acid detergent fibre does not include hemicelluloses, and crude fibre analysis does not quantitatively recover hemicellulose and lignin. The term crude fibre is nowadays very rarely revered to in scientific ruminant research papers. Neutral detergent fibre (NDF) is the method that best separates structural from non-structural carbohydrates in plants. Neutral detergent fibre (NDF) measures most of the chemical compounds generally considered to comprise fibre (NRC, 2001). On average the NDF fraction (proportions of cellulose, hemicellulose, and lignin) is less digestible than non fibre carbohydrates (NFC); therefore, the concentration of NDF in feeds or diets is negatively correlated with energy concentration (NRC, 2001). This proportions of cellulose, hemicellulose, and lignin effects the digestibility of the NDF fraction meaning that feeds with similar NDF concentrations will not necessarily have the same net energy values for growth or lactation. The optimum NDF in any

ration is a balance between net energy requirements, physical, and physiological regulators on intake, and these aspects will be discussed in section 2.4.2.

2.3.1 Effective NDF (eNDF) and physical effective NDF (peNDF)

Effective neutral detergent fibre (eNDF) is described as the percentage of NDF remaining on a 1.18mm screen after dry sieving (Smith and Waldo, 1969, Mertens, 1985). This led Sniffen *et al*. (1992) to develop eNDF values for most feedstuffs. "Effective NDF" is the percentage of the NDF effective in stimulating chewing and salivation, rumination, and rumen motility (Beauchemin, 1991; NRC, 1996). The importance of stimulating salivation for rumen buffering is well documented (Beauchemin, 1991). Additional factors such as total grain intake, digestion rates, processing of grains (whole shelled maize vs. dry rolled maize) also have an indirect influence on rumen pH and is not accounted for in the eNDF system (NRC, 1996). According to the Dairy NRC (2001) eNDF is defined as the sum total ability of NDF in a feed to replace the NDF in forage or roughage in a ration so that the percentage of milk fat is maintained.

The term physically effective NDF (peNDF) originated from early researched work with dairy cattle and it revered to the physical characteristics of NDF (primarily particle size) that stimulated chewing activity and rumen functions (osmolality, and motility). Mertens (1997) developed the peNDF system with assigning physical effective factors (PEF) to feeds based on the chewing activity the feeds stimulated. Physical effective factor values were assigned for a large variety of feeds, example: PEF value of long hay is 1; coarsely chopped hay and silages are 0.9 and finely chopped roughages 0.7 – 0.85. These values were determined for a variety of roughages. The problem with assigning factors is one of how to analyze, quantify and replicate these factors. Mertens (1997) referred back to original work of Mertens (1985) proposing that dry sieving feeds on a sieve with an aperture of 1.18mm will be an effective and routine laboratory method for analyzing eNDF. In practice almost all forage or roughage in feedlot and dairy diets are processed (chopped, finely chopped, ensiled or pelleted) and the extent of processing might alter the eNDF values theoretically assigned to them. In the study conducted by Defoor *et al.* (2002), research results provided a preliminary indication that depending on the roughage sources evaluated, roughage NDF content and(or) roughage NDF from particles larger than 2.36mm might provide a useful index of roughage value in high-concentrate finishing diets. Lammers *et al.,* (1996) developed a three screen (>19mm, 8-19mm, <8mm) sieve for evaluating feed particle sizes. With the addition of a fourth sieve (<1.18mm) it is currently known as the Penn State Forage Particle Separator (PSPS) which is widely used in practice to evaluate particle size distributions of roughage in dairy rations (Kononoff *et al*., 2003). The general lack of standard evaluation for effective fibre measurements, limits the application of the peNDF concept but Mertens (1997) peNDF system is a progressive step towards a better quantification of fibre constituents (chemical, and physical attributes).

2.3.2 Setting minimum standards for eNDF and peNDF requirements in feedlot diets

Recommendations published in the NRC (1996) suggested that 25% eNDF may be required for beef cattle to maintain a ruminal pH acceptable for maximum fibre digestion and microbial growth. Fox & Tedeschi (2002) recommended peNDF requirements of between 7 and 10% of ration DM for high concentrate diets but if the goal is to optimize forage utilization the minimum requirements for peNDF is set at 20% of DM. The recommended 7% in finishing diets is based on the eNDF level predicted by the equation derived by Pitt *et al.* (1996) required to maintain rumen pH above 5.7.

Ruminal pH = 5.46 + 0.038 (% eNDF), eNDF < 26.3%

(r^2 = 0.52, P < .001)

Threshold pH of 5.7 was regarded as the ruminal pH where cattle usually reduce DM intake (Britton and Stock, 1989).

2.4 APPLICATIONS FOR THE USE OF PHYSICALLY EFFECTIVE FIBER IN FEEDLOT DIETS

2.4.1 The digestion of neutral detergent fibre in mixed diets

Percentages of fermented fibre carbohydrates (FC) and non fibre carbohydrates (NFC) in the rumen vary, depending on digestion and passage rates. Growth rates of bacteria digesting available FC and NFC depended on rumen pH, which in turn can be predicted from the NDF and eNDF in the diet. Effective NDF stimulates chewing and rumination, thereby increasing rumen pH through buffering by saliva (Beauchemin, 1991). In the Cornell Net Carbohydrate and Protein System, Russel *et al.* (1992) categorized the micro organisms into those fermenting FC and those fermenting NFC. Fibre carbohydrates micro organisms ferment cellulose and hemicelluloses and NFC micro organisms ferment starch, pectin's, and sugars. Fibre carbohydrates micro organisms grow slower than NFC micro organisms and have different rumen turnover rates as well as different maintenance requirements (*Streptococcus bovis*, a primarily starch fermenter, has 6 times the maintenance requirements of *Fibrobacter succinogenes*, a primarily fibre fermenter). The extent of replacement of roughage sources for concentrates in mixed diets will have adverse effects on the digestibility of the NDF source due to depression of FC micro organisms. To account for variability in ruminal and total tract digestibility of the NDF, Firkins (1997) used multiple regression analysis to indicate that non-forage NDF percentage in the diet had about two-thirds the positive response on total tract NDF digestion than what forage NDF percentage had.

In the study conducted by Poore *et al.* (1990), they concluded that total tract digestibility of NDF was not altered, but digestibility of potentially digested NDF decreased (P<.05) from 92 to 48% as concentrate level in the experimental diets increased from 30 to 90% of DM. This rather large depression in dietary NDF digestion could be explained by the large contribution of grain NDF (54%) to total dietary NDF in their study. Calculated ruminal digestibility's in NDF for individual ingredients decreased (P<.05) by 72, 57, and 34% for wheat straw, Lucerne hay and grain respectively when concentrate level was increased to 90% of the diet. This confirmed that depression in digestibility for fibre sources was more severe than for grain sources when concentrate level in the diet increased. This also reflected on the passage rates of low quality roughage sources, being severely depressed at 90% concentrate level with little or no change in passage rates for grains (Poore et al., 1990). In conclusion, grain contributed a large portion of NDF in mixed diets for feedlot cattle and the digestibility of this NDF source is much higher than for roughage source NDF but it also accounted for much of the potentially digestible fibre leaving the rumen.

2.4.2 Dry matter intake, physical and physiological responses to effective fibre

Dry matter feed intake and ultimately energy intake is influenced by physical fill and physiological mechanisms determined by the target animal. The concept of intake regulation was best described by Mertens (1994). This author suggested neutral detergent fibre can be used as indicator for the "filling effect" or physical fill by fibre sources in the reticulorumen. The theory suggested that physical fill (rumen filling capacity) limits long-term energy intake by ruminants up to a point, after which physiological factors (high VFA concentration, and associated low rumen pH) limits intake. Results published in the (NRC, 2001) suggested that dairy diets with 44% NDF would most likely limit energy intake. This implied that if adding bulk feedstuffs high in NDF, to a diet already on its upper limit of NDF concentration, decreased DMI and energy intake can be expected. The opposite of this theory applied to feedlot diets where diets contained between 4.5 to 13.5% roughage (DM basis) for finishing diets (Galyean and Gleghorn, 2001). In this case intake regulation would be more affected by physiological mechanisms as physical fill would be a negligible factor due to less bulky feeds (roughage) high in NDF. Adding bulk fibre (feeds high in NDF) to diets already low in NDF would stimulate increased DMI with little change in energy intake. Low percentage increases of roughage in high-concentrate diets may also prevent digestive upsets and maximize energy intake by feedlot cattle. This compensation effect through increasing DMI was possible up until the point where physical fill became the restriction to DMI. Galyean and Defoor (2003) suggested that below the restriction point, relatively small increases in dietary fibre from roughage source may stimulate DMI to the point where even total daily energy intake can be increased. This increased DMI can probably also be explained by an "energy dilution" effect since most roughage sources were much lower in energy values than concentrates. This meant that animals may have to consume more of the less energy dense diet to keep up with their daily metabolic energy demand. Under these energy dilution

circumstances, dietary fibre level increase is usually large in proportion to the previous dietary fibre level. Differences in DMI due to addition of small amounts of roughage might be a result of altered digesta kinetics, altered rumen fermentation, and rumen or metabolic pH changes rather than from energy dilution effects. In conclusion, source and level of roughage replacements and additions to finisher diets with low level fibre concentrations, may have very different responses on DMI, total daily energy intake and rumen fermentation patterns.

2.5 RUMEN FERMENTATION ACID PRODUCTION AND RUMEN pH IN RELATION TO EFFECTIVE FIBRE

2.5.1 The effects of dietary eNDF content on ruminal pH and fermentation

Maintaining a high extent of rumen fermentation is desirable for maximizing total starch and fibre digestion as well as microbial amino acid production. Using the CNCPS model (Fox *et al.*, 1995) an evaluation of experimental data obtained from various researchers confirmed this statement. Declined rumen pH as a result of high rate of ruminal starch digestion, caused a reduction in microbial protein synthesis (Russell *et al.*, 1992), a reduction in cell wall digestion (Pitt *et al.*, 1996) and a higher incidence of acidosis (Owens *et al.,* 1996). The level and type of grain as well as the degree of processing of grain will determine the rate of starch fermentation and the incidence of sub-clinical acidosis (Owens *et al.,* 1996). Ruminal absorption of VFA is associated with rumen epithelium metabolism as well as uptake and transport into the bloodstream. Rumen epithelium is more permeable to the un-dissociated state of VFA's than the dissociated state of VFA's. This implies that with an incremental decrease in ruminal pH, increased absorption of acids by rumen epithelium will take place. Furthermore several other factors such as: rumen surface area, acid concentration and acid type may determine absorption rates (Van Soest, 1994). Although individual VFA fractions were not accurately predicted by developed prediction equations for the CNCPS model, a literature data review by Pitt *et al*., (1996) showed effective NDF to have better correlation with ruminal pH than forage or dietary NDF. Galyean and Defoor (2003) suggested that although some differences in rumination time and ruminal pH have been noted among certain roughage sources and levels, statistical relationships between effective NDF as defined by NRC (1996) and ruminal pH are not strong. Buffering capacity of roughage sources may or may not account for differences in ruminal and metabolic acid loads. Inherent buffering capacity relates to the cation exchange capacity (CEC) of different feeds as done by McBurney *et al*. (1983), and this was measured by the copper or praseodymium uptake of NDF (McBurney *et al.*, 1986). These research workers categorized feedstuffs, with relatively high NDF and high CEC values to have the highest buffering capacity of all feeds tested. Several feeds were tested by the author and hays had somewhat lower buffering capacity, silages and

straws, however were rather low in their buffering capacity compared to beet pulp. Some roughage also contained chemical substances like slaframine which stimulates saliva production (Froetchel *et al*., 1986). The contribution of these substances to total buffering capacity in feedlot finishing diets remains to be determined. Allen (1997) indicated that the direct buffering effect from feeds alone comprises a small portion of the buffering capacity resulting from saliva flow and that the inherent buffering capacity of roughages in typical dairy diets high in NDF is small. Galyean and Abney (2006) argued that in typical finisher diets with high concentrate, low roughage concentrations, the effect will even be more negligible.

2.5.2 The effect of pH on lag time and NDF digestibility

Initiation of digestion can be limited by preferential substrate use, pH effects or specific nutrients required by bacteria or a combination of these factors. Lag time, or time required for attachment of microbes to forage particles, is known to increase with an incremental increase in starch supplementation of forage diets (Mertens and Loften, 1980). Shriver *et al*. (1986) evaluated the effect of pH during continuous culture fermentations and recorded a decrease in ammonia nitrogen, NDF digestibility and the proportion of microbes attached to fibre particles when rumen pH is between 5.8 and 6.2. This decreased initiation of digestion on fibre due to low pH might be one reason. The initial response by microbes not to digest feed particles, which occurred at pH 5.8, may be due to decreased microbial attachment to feed particles. Low ammonia nitrogen and decreased protein digestion may further reduce fibre digestion through an effect on the growth of cellulolytic microbes (Shriver *et al*., 1986).

2.5.3 The effect of NDF source on fermentation end products

Whether NDF from forage or non-forage and/or the concentration thereof in the diet affects absorption of end product acids from the rumen, or changes the acidity and uptake in the intestines remains to be determined. Galyean and Defoor (2003) suggested that it seemed unlikely that forage NDF would alter direct absorption rate of VFA in high concentrate feedlot diets, but forage NDF might rather change digesta kinetics, water flux and post ruminal absorption of volatile fatty acids. To bring this statement into context one has to consider the mechanism of removal of fermentation acids from the rumen as best described by Allen (1997). In general, VFA absorption increases as pH decreases. Acetic, propionic, and butyric acids are absorbed at similar rates at a neutral pH; but as pH decreases, absorption rates increase as molecular mass increases. Although the major VFA's have similar acid dissociation constants, they have different effects on ruminal pH because of differential rates of absorption. This means that acetic acid has a greater effect on reducing ruminal pH than do propionic acid, which is greater than butyric acid; the magnitude of these differences increases as ruminal pH decreases. Flux of absorbed acids by rumen epithelium depends on the surface area for absorption. Allen (1997) also noted that adaptive changes in

rumen papillae length to increase surface area, due to diets varying in rumen digestible organic matter, might be an important factor affecting the susceptibility of animals to suffer rumen acidosis. Fermentation acid flow post ruminally are associated with the larger liquid fraction. Some acids are associated with the smaller particulate pool. The fraction of acids absorbed post ruminally increased as the liquid passage rate increases and as ruminal pH increases (Allen, 1997). This led Galyean and Defoor (2003) to the conclusion that forage NDF are more likely to affect the digesta kinetics and position of absorption rather than absorption rate of fermentation acids. For this very same reason higher forage NDF would result in higher DMI and higher water intake that will have a diluting effect and ultimately leads to a decrease in acid load (Galyean and Defoor, 2003). Acetate: propionate (A: P) ratio is highly correlated with the amount of forage NDF in the diet, being a minimized ratio of A: P when diets are low in forage and high in fermentable starch (Armentano and Pereira, 1997). Changes in propionate concentration change with increases in osmolality of the rumen due to excessive and rapid fermentation of starch to VFA. Thus, decreasing dietary NDF concentration and increasing fermentable dietary starch concentration, is associated with increased propionate production and absorption and may be responsible for lower DMI (Galyean and Defoor, 2003).

2.6 CHARACTERISTICS OF RUMINAL DIGESTA, DIGESTA FLOW, AND SITE AND EXTENT OF DIGESTION

2.6.1 The interaction between non-forage fibre sources (DFFS) and dietary forage on the ruminal mat consistency

Source, amount and physical characteristics of dietary forage can interact with non-forage fibre sources by influencing ruminal, and total tract fibre digestion and passage rate of particles when high levels of non-forage fibre sources replace forage fibre sources. The highest dry matter (DM) content in the reticulorumen is located in the dorsal strata of the rumen. The physical nature of the contents in the rumen is affected by the dietary concentration of fibre. The majority of this DM is composed of large particles, but fine particles with a mean length < 1.0mm occur in high concentrations over a 24 h period in the dorsal rumen. Through filtration and mechanical entrapment of particles, the rumen mat functions to retain potentially escapable digestible fibre. Lechner –Doll *et al.* (1991) postulated that the probability of a feed particle exiting the rumen is primarily determined by its particle size and density. Kaske and Von Englehardt, (1990) found that 1mm plastic particles with a density of 1.44 exited the rumens of sheep 24 times faster than those with specific gravities of 0.92 to 1.03. Non-forage fibre sources have the potential to replace forage however their small particle size (≤ 1mm) and high specific gravity (ranges between 1.4 and 1.5) may facilitate rapid removal from the reticulorumen (Siciliano-Jones and Murphy, 1991). Ultimately, these non-forage fibre sources diminishes in value as fibre, as rumen degradability of their NDF fraction diminishes due to ruminal escape. Grant (1997) concluded that the amount of forage in the diet and

forage particle size interact with the substituted non-forage fibre source to determine the net impact on rate of ruminal digestion and passage of non-forage fibre. This author postulated a model for replacement of forage fibre with non-forage fibre, emphasizing the interactions among forage level and particle size, and on dietary concentration of non-forage fibre on ruminal fibre disappearance. The model implies that if high levels of NFFS replace forage, it would result in a lower dietary forage level, therefore care should be taken to ensure a particle size of sufficient length to stimulate rumination, maintaining optimal pH, and retain small feed particles. The Grant (1997) model is shown in Figure 1 and demonstrates the potential interactions among forage level and particle size as well as the interactions of the amount of non-forage fibre on ruminal fibre digestion and passage rates. The diagram implicates diets high in non-forage fibre, small particle size and low forage levels will result in lesser entrapment of particle size in the rumen, fibre passage rate will increase, fibre digestion rate will decrease and potentially result in lower rumen pH levels.

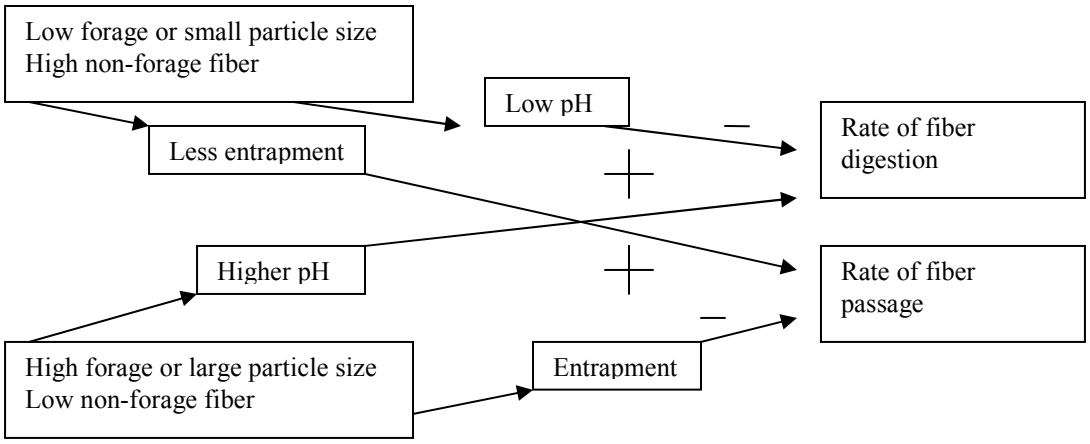

Figure 1. Potential interactions among forage level and particle size, and the effect of the amount of non-forage fibre on ruminal fibre digestion and passage rates (Grant, 1997)

By-product feeds like brewer's grains, gluten feed; wheaten bran and hominy chop are common ingredients in diets used in the South African feedlot industry. Non-forage fibre sources like hominy chop often replace starch with positive effects on fibre digestion negating the negative effects of excessive starch fermentation and associative low fibre digestion due to an unfavourable pH in the rumen. Firkins (1997) indicated that the rates of NDF digestion from non-forage fibre sources are similar to or slower than those from forage sources, but passage rates of non-forage fibre sources are faster than those of forage sources. Changing passage rates of dietary components from the rumen relates to changes in DMI. Thus, if NDF from a roughage source increases passage rates of grains, less rumen fermentation would occur and one would expect a shift in fermentation from the rumen to digestion in the intestines. This change in digestion

location of starch is not necessarily negative since it reduces acid load in the rumen and stimulates higher DMI; however, when considering efficiency, unfermented starch digestion in the intestines are counterproductive since the ability for starch digestion in the intestines of ruminants are limited (Huntington, 1997).

2.6.2 Particle size, hydration kinetics of particles and mean retention time in the rumen

The nutritive value of forages consumed by ruminants is influenced by both the rate of degradation as well as the rate of removal or outflow from the rumen. Faichney (1983) concluded that feed particles exist in continuum sizes, from very small to very large (<1mm and >1cm). These feed particles are reduced in size by rumination and by microbial degradation. All these particle sizes can be categorized by the rate at which they have the probability to exit the rumen. Those particles in the category for low probability of removal from the rumen are those with a critical size greater than a sieve pore size of 1.18mm. Faichney (1983) described the "filter bed" effect as separation method in the rumen for larger particles and the associative fluid phase. Small particles (<0.15) associates with the fluid phase and will be removed from the rumen at the same rate. Functional density of digesta particles in the rumen can be defined as the sum of all factors influencing its buoyancy, including structural components of the particle, fluid and gas inside the particle as well as attached gas bubbles (Lechner- Doll *et al.*, 1991). Wattiaux *et al.* (1992) investigated the kinetics of hydration of ground hay and silage particles (2mm diameter). The researchers demonstrated that the water holding capacity might be an important property influencing functional density of forage particles and subsequent ruminal retention time of forage particles. Preservation of forages as silages reduces gas volume and liquid retention properties of particles, both of which contribute to greater specific gravity and possible shorter ruminal retention time.

2.7 RUMINAL ACIDOSIS AND DIETARY FIBER

Acidosis is the decrease in the alkali (base excess) in body fluids relative to the acid (hydrogen ion) content (Stedman, 1982). Although pH of body fluids is buffered by bicarbonate, pH of body fluids may or may not be depressed during acidosis, depending on the degree to which bicarbonate compensation is possible. A sudden fall in blood bicarbonate concentration can disturb central nervous system functions even if blood pH is not depressed. Clinical diagnosis of acidosis requires blood pH to fall below 7.35 but other clinical symptoms such as low ruminal pH, anorexia, diarrhea and low or variable DM intake are routine diagnostic indication of acidosis in feedlot cattle (Owens et., 1998). A detailed description of the etiology of ruminal and systemic acidosis has been described in reviews by Huntington (1988), Nocek (1997) and Owens *et al.* (1998). Acidosis is a term used collectively for a description of digestive disturbances of the rumen and intestines. Acidosis is currently the most misdiagnosed phenomenon in

feedlots with several forms and degrees of this metabolic disturbance being observed. These forms includes acute, chronic (subclinical), and subliminal types. Animals exhibit acute acidosis as an overt illness following consumption of readily fermented carbohydrates in amounts sufficient to reduce ruminal pH. Animals with chronic acidosis may exhibit low or variable feed intake and reduced performance but may not appear to be sick. Ruminal pH of 5.6 and 5.2 are often being used as benchmarks for chronic and acute acidosis respectively (Cooper and Klopfenstein, 1996). However the length of time for ruminal pH to be on or under this threshold pH will determine the severity of the condition. Subacute ruminal acidosis (SARA) indicators appeared to be daily episodes or periods below ruminal pH of 5.6 for at least 3 hours (Cooper *et al*., 1999; Gozho *et al*., 2005). Additional symptoms are non specific and may include diarrhea, laminitis and liver abscesses (Nocek, 1997).

Ruminal pH is affected by the fibre concentration of the diet and the balance between the production of fermentation acids and secretion of buffers (Krause *et al.,* 2002). Decreasing the diet crude fibre, NDF or ADF generally decreased rumen pH (Erdman, 1988; Kolver & De Veth, 2002). Calsamiglia *et al*. (2002) found that a ruminal pH kept at a constant 5.7 had a negative impact on digestibility of apparent DM, NDF and ADF, lowered total and branched-chained VFA concentrations, and lowered acetate but incremental propionate proportions compared to when ruminal pH was kept at constant 6.4. Readily fermentable carbohydrates in general decrease ruminal pH due to rapid microbial fermentation resulting in a subsequent higher production of VFA concentrations (Strobel & Russel, 1986). Furthermore, rumen pH was related to VFA concentration (Erdman, 1988; Stokes *et al.*, 1991) and ruminal pH decreased in the case when a decreased absorption rate of VFA was present (Owens *et al.*, 1998).

2.8 VOLATILE FATTY ACID PRODUCTION, LACTATE PRODUCTION AND UTILIZATION DURING ACIDOSIS

The balance between lactate producing and lactate utilizing bacteria determines whether lactate accumulates or not. Most lactate utilizing bacteria are sensitive to low pH, whereas lactate producers are not. Under anaerobic conditions, pyruvate is converted to lactate for production of NAD, used in glycolysis. Lactate does not accumulate under normal conditions in the rumen and the level of concentration is usually below 5 µM, however levels exceeding 40 mM are an indication of severe acidosis (Owens *et al.,* 1998). When lactic acid accumulated after induced acidosis in lambs, blood pH decreased from 7.44 to 7.2 within 30 hours after onset, packed cell volume increased, bicarbonate and calcium decreased, and blood lactic acid increased 6 fold (Huntington and Britton, 1979). In the same study it was reported that two thirds of the total lactic acid where in the D-lactic form. The spiralling process of lactic acidosis described by Russel and Hino (1985) as documented by Nocek (1997) is illustrated in Figure 2. The sequence is triggered by a carbohydrate insult (for example a 90- 100% concentrate diet), followed by

rapid fermentation and accumulation of volatile fatty acids. It also explains the role of D and L-lactic acid in inducing systemic acidosis.

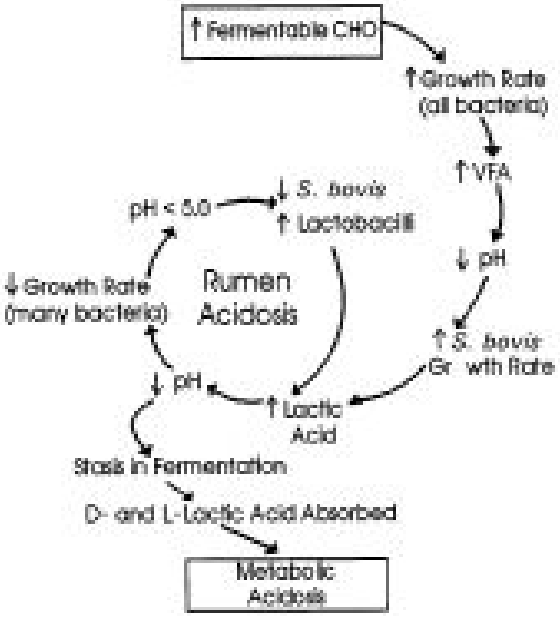

Figure 2. An illustration of the events associated with the induction of acute ruminal lactic acidosis. (Nocek, 1997).

When ruminal pH drops below 5, growth rate of bacteria slow down and lactic acid producers like *Streptococcus bovis* continue to produce lactic acid. Once the physiological conditions in the rumen have overwhelmed the microbial populations like *M. elsdinii*, *P. shermanii* and *S. ruminantium* to sequester lactic acid, absorption into the bloodstream results in systemic acidosis. The two types of lactic acid isomers namely L-lactate and D-lactate have different turnover rates, metabolism and elimination pathways. Between 40 and 65% of L-lactate is derived from glucose (Reilly and Chandrasena, 1978; Owens *et al*., 1998) and roughly 20% of the lactate that enters the system is converted to glucose with 30 to 50% being oxidised to CO_2. The Cori cycle converts L-lactate to glucose and this represent 5-10% of total glucose produced while L-lactate is an important precursor for fatty acid synthesis as well as glucogenesis. About 16 -38% of the acetate used for fatty acid synthesis originates from lactate and this conversion is particularly important at high intakes during which the turnover rate of lactate is much more rapid than acetate (Giesecke and Stangassinger, 1980). The volatile fatty acids do not usually accumulate at high enough concentrations to drastically reduce rumen pH but when rate of production exceeds rate of absorption, depression of ruminal pH below 5 can be observed even without the accumulation of lactic acid (Owens *et al*., 1998). The metabolism of the rumen wall and the liver may be compromised during acidosis and the situation gets complicated with the liver being faced with L-lactate from muscular tissue metabolism as well as D- and L-lactate from the digestive tract. In the case of a well adapted larger liver,

capacity to metabolise lactate is better than smaller un-adapted livers and the chances that these animals will exhibit blood acidosis is slim. The proportion of the two lactate isomers change as ruminal pH decrease, with D-lactate constituting about 20% of the total lactic acid concentration at pH 6 and elevates to 50% at ruminal pH less than 5. The metabolism of these isomers as well as the elimination from the blood stream was well documented in reviews by Nocek (1997) and Owens *et al.* (1998).

2.9 CONTROL OF RUMINAL pH

Increased ruminal input of buffers such as bicarbonate from the diet and or saliva, or ammonia from degraded protein or non-protein nitrogen (NPN), will help prevent a depression in ruminal pH. In addition to buffers, absorption of VFA, by removing unionized acids due to an exchange of ionized VFA for bicarbonate during the absorption process, will aid in maintaining ruminal pH at neutral (Owens *et al.,* 1998). Allen (1997) found ruminal pH very responsive to chewing behaviour, with pH decreases post feeding and pH increases occurring with rumination. Ruminal pH declines following meals, with the drop in pH depending on the initial pH (Allen, 1997; Meakawa *et al.,* 2002). Approximately half the bicarbonate entering the rumen comes from saliva during eating and rumination, the other half enters the rumen in exchange for ionized acids being absorbed (Owens *et al*., 1998). With concentrate diets, reduced salivary input must be counter balanced with higher proportions of bicarbonate from the blood. Ruminal pH declines steadily for a period of 4-6 hours post feeding with lowest pH usually also around 4- 6 hours post feeding (Lindberg, 1981; Madsen & Hvelplund, 1988). The rate of ruminal pH decline was faster as meal size increased and dietary NDF concentration decreased (Dado & Allen, 1993; Meakawa *et al*., 2002). Haaland *et al.*, (1982) reported pH at maintenance to be 6.57 and at twice maintenance to be 6.35 while Madson (1986) found doubling feeding level resulted in a ruminal pH change from 6.59 to 6.47. Increased ruminal degradation is desirable to maximize microbial protein production and energy intake, but the increase of fermentation acids must be compensated for by either increasing the dietary NDF or by salivary secretion of buffers.

Osmotic pressure through membranes is regulated by the relative concentration of dissolved materials across membranes. This mechanism of osmotic pressure pulls water or push water across membranes. High concentrations of dissolved materials in the rumen, pulls water from the body. Minerals, VFA, lactate, and glucose are primary solutes in ruminal fluids while dissolved proteins contribute to osmotic pressure in the blood. High rumen osmolality from elevated concentrations of glucose and acids cannot be readily prevented. During high rumen osmolality situations, VFA absorption across the rumen wall decreases and a rapid influx of fluid from the body can cause tissue damage in the rumen wall and intestines which lead to ulcerations and a potential entry spot for pathogens directly into the blood stream.

Control through diet manipulation or water is possible by altering osmotic pressure through minerals (sodium, potassium and chloride) or ammonia release. Reducing these mineral salts may reduce ruminal osmolality slightly while increasing the saliva output. This may also contribute to a decrease in ruminal osmolality (Owens *et al*., 1998). Water intake may have little or no effect on rumen osmolality since it may partially flush past the rumen (Garza and Owens, 1989).

The mechanisms involved in controlling acid absorption from the rumen are complex and depends on the several factors. Rapid removal or absorption of acids from the rumen to avoid ruminal acid overload and increased osmolality depends on the potential of the system (blood, liver) to handle this additional influx of acids. Temporarily reducing absorption rate of acids across the rumen wall will decrease ruminal pH and increase osmolality and will depend in contrast on the state of the rumen to handle the acid load. Acid absorption rate is reduced by increasing ruminal pH. Higher bicarbonate input from the diet or saliva will increase the ruminal pH and in turn stimulates the utilization of lactate in the rumen. Higher ruminal ammonia concentrations from higher dietary protein or urea concentrations often are associated with higher ruminal pH and ruminal buffering capacity (Haaland *et al.,* 1982) although only 10 to 15% of the VFA produced in the rumen, can potentially be neutralized by ammonia release. Protein sources including NPN that release ammonia gradually may be beneficial to keep ruminal pH at neutral. Blood VFA concentrations remain low due to rapid metabolism of VFA to glucose or carbon dioxide. Only acetate normally reaches the peripheral blood stream. Butyrate is converted to beta-hydroxybuterate during absorption through the rumen wall and all the propionate is converted to glucose by the liver. Blood pH control is a balance between cation and anion exchange. Increasing the base excess, through altering the cation-anion balance of the diet, should aid in preventing acidosis. Ammonium salts are excreted to counteract an acid load. During chronic acidosis, elevated levels of ammonia may be required to stabilise blood pH (Owens *et al.,* 1998).

From this review it is clear that there are still many questions with regards to the interaction between different forage and non-forage fibre sources, their inclusion levels, their particle sizes and the resulting effects on rumen fermentation patterns. It is also clear that metabolic disturbances like acidosis can be minimized through manipulation of diets by varying levels of NDF from forage or long fibre. Some of these aspects will be addressed and evaluated by means of 2 research experiments. In experiment 1 the effect of four different roughages in combination with hominy chop on rumen fermentation was investigated. In experiment 2 the same roughages, but in combination with a different energy source namely dry rolled maize, was investigated.

2.10 HYPOTHESIS

The hypothesis for this experimental work is:

H_0: That different roughage sources in combination with a single non-fibre carbohydrate will result in no difference in fermentation characteristics and ruminal pH.

And

H_1: That different roughage sources in combination with a single non-fibre carbohydrate will result in different rumen fermentation characteristics and ruminal pH.

CHAPTER 3.

3. MATERIALS AND METHODS

3.1 Introduction

In order to address some of the aspects documented in the literature review, a research trial was design to test and evaluate the fermentation characteristics and the consequent effects on rumen pH of some practical finishing diets commonly used in South African feedlots. This trial design included cannulised bulls from a commercial feedlot used in a 4 X 4 Latin squire design. Finishing diets were formulated using four different roughage sources in combination with two energy sources. The research was conducted by performing two separate experiments to evaluate the effect of the four different roughage sources in combination with firstly hominy chop, and secondly dry rolled maize as energy sources on the measured rumen fermentation parameters.

3.2.1 Experimental animals

Four Beefmaster steers from Manjoh Ranch with the average age of eight months were used in this trial. The animals, weighing 270kg (± 15kg) were cannulised at Manjoh Ranch upon arrival. Animals were surgically fitted with 100mm diameter cannulas (Bar Diamond Inc., Parma, ID, USA) by the veterinarians from the Faculty for Veterinary Science University of Pretoria. The procedures and experimental design for this research project was submitted and approved by the animal use and care committee as well ethics committee of the University of Pretoria. Cannula wounds on all the experimental animals were disinfected (Figure 3.1) and treated once daily and animals were monitored for temperature and feed intake during the first seven days after cannulation procedures. Animals were adapted for a period of 21 days on the commercial feedlot starter diet at Manjoh Ranch and then changed over to a grower diet for 20 days in the feedlot. The Experimental animals were then transported to the Hatfield experimental facility of the University of Pretoria for the final finishing phase using the experimental finisher diets. Animals were individually penned for the experimental period in a facility that matched commercial feedlot conditions. Body weight was recorded before feeding at the beginning of each experiment and after each experimental period ended.

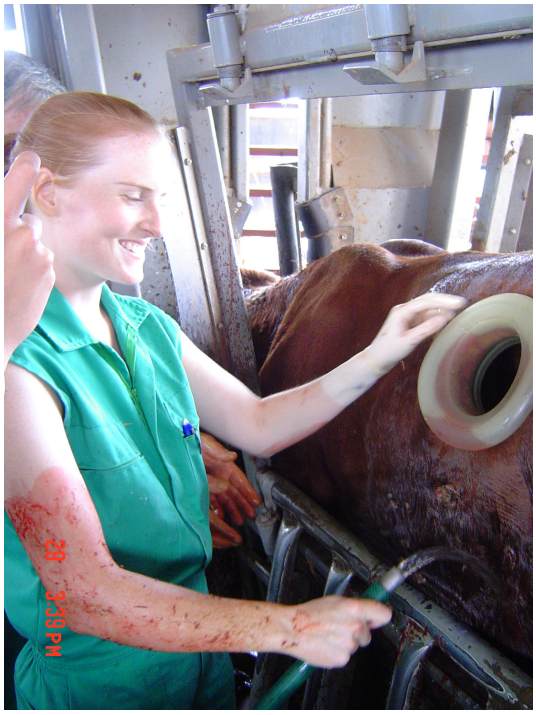

Figure 3.1 Demonstrate the disinfecting and cleaning of wounds after the cannulation procedure.

3.2.2 Experimental design

The experimental design was a 4 x 4 Latin square (Table 3.1 and Table 3.2) repeated in two experiments. Experimental animals were randomly assigned to fit the Latin square design in both experiments. In order to substantiate between the effect of different roughage sources and energy source interaction on rumen fermentation, the experimental procedure were divided into two separate experiments, namely experiment 1(four different roughage sources in combination with hominy chop as a major source of non fibre carbohydrate source), and experiment 2 (four different roughage sources in combination with dry rolled maize as major source of non fibre carbohydrate). Each experimental period consisted of 14 days, 13 days for adaptation to the experimental diet followed by a 24 hour period in which rumen fermentation parameters and ruminal pH were measured.

EXPERIMENT 1

The experimental design was a 4 x 4 Latin square. Each animal received a diet differing in roughage source during each of the four periods. The four different roughage sources were used in each diet with hominy chop as a sole en source of non fibre carbohydrate source. These roughage sources and energy source are commonly used in combination in South African feedlots. The four experimental diets were:

DIET 1 = WSHC (wheat straw, hominy chop diet)

DIET 2 = EHHC (*Eragrostis curvula* hay, hominy chop diet)

DIET 3 = CHHC (cottonseed hulls, hominy chop diet)

DIET 4 = MSHC (maize silage, hominy chop diet)

EXPERIMENT 2

The experimental design was a 4 x 4 Latin square designed experiment. Each animal received a diet differing in roughage source during each of the four periods. The four different roughage sources were used in each diet with dry rolled maize as a sole non fibre carbohydrate source. These roughage sources and energy source are commonly used in combination in South African feedlots. The four experimental diets were:

DIET 1 = WSDRM (wheat straw, dry rolled maize diet)

DIET 2 = EHDRM (*Eragrostis curvula* hay, dry rolled maize diet)

DIET 3 = CHDRM (cottonseed hulls, dry rolled maize diet)

DIET 4 = MSDRM (maize silage, dry rolled maize diet)

The experimental diets are listed in Table 3.1 and Table 3.2

Table 3.1 Experimental diets fed in Experiment 1

	Animal number			
Period	1	2	3	4
1	WSHC[1]	EHHC	CHHC	MSHC
2	CHHC[1]	WSHC	MSHC	EHHC
3	EHHC[1]	MSHC	WSHC	CHHC
4	MSHC[1]	CHHC	EHHC	WSHC

[1] Experimental diets: WSHC (wheat straw, hominy chop); CHHC (cottonseed hulls, hominy chop); EHHC (*Eragrostis curvula hay*, hominy chop); MSHC (maize silage, hominy chop)

Table 3.2 Experimental diets fed in Experiment 2

	Animal number			
Period	1	2	3	4
1	WSDRM[1]	EHDRM	CHDRM	MSDRM
2	CHDRM[1]	WSDRM	MSDRM	EHDRM
3	EHDRM[1]	MSDRM	WSDRM	CHDRM
4	MSDRM[1]	CHDRM	EHDRM	WSDRM

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); MSDRM (maize silage, dry rolled maize)

3.2.3 Experimental diets

Experimental diets were formulated using the SPESFEED Express version 2.6 formulation program (Spesfeed (PTY) Ltd., Unit 6, Bentley Office Park, 67 Wessels rd., Rivonia, South Africa) to be representative of diets fed by commercial feedlots in South Africa. The ingredients and chemical composition of the experimental diets are presented in Tables 3.3 and 3.4 for Experiment 1 and Experiment 2 respectively. Experimental diets where manufactured by the SIS Feed mill in Middelburg, Mpumalanga, South Africa. Maize silage was provided by Chalmar Beef, Bapsfontein once per week and stored in special airtight container bags to guarantee freshness. All maize silage diets where prepared fresh daily at the experimental facility by weighing of concentrate and silage and mixing thoroughly in the feed bunk for each individual animal.

Table 3.3 Ingredient and chemical composition (%DM) of the four experimental diets (Experiment 1)

	Diets[1]			
Item	WSHC	EHHC	CHHC	MSHC
Ingredient				
Maize Silage				15.0
Eragrostis curvula hay		7.5		
Cottonseed hulls			7.5	
Wheat straw	7.5			
Hominy chop	67.3	67.9	70.7	57.9
Molasses	7.2	9.0	6.2	9.0
Cottonseed oilcake	2.5	2.5	2.5	2.5
Wheat bran	12.7	10.3	10.2	12.9
Limestone	1.8	1.8	1.8	1.8
Salt	0.2	0.2	0.2	0.2
Urea	0.8	0.8	0.8	0.8
Trace mineral Premix[2]	0.1	0.1	0.1	0.1
Estimated chemical composition (DM basis) [3]				
DM (%)	86.5	86.1	86.5	70.5
Crude Protein (%DM)	13.7	13.7	13.7	13.7
UIP (%DM)	5.1	4.9	5.2	4.6
DIP (%DM)	8.6	8.6	8.5	9.1
Urea (%DM)	0.8	0.8	0.8	0.8
TDN (%DM)	77.7	79.0	78.6	78.9
ME (MJ/kg)	12.3	12.4	12.4	12.4
NEm (MJ/kg)	8.4	8.4	8.4	8.4
NEg (MJ/kg)	5.6	5.6	5.6	5.6
Roughage (%DM)	7.5	7.5	7.5	7.5
Crude Fibre (%DM)	11.0	10.5	11.5	10.1
NDF (%DM)	32.9	31.0	33.4	29.9
eNDF (%DM)	9.1	8.0	9.8	7.5
Fat (%DM)	7.9	8.0	8.1	7.2
Starch (%DM)	26.7	27.0	27.6	28.0
Ca (%DM)	0.7	0.7	0.7	0.7
P (%DM)	0.6	0.6	0.6	0.6
Total Salt (%DM)	0.25	0.25	0.25	0.25
Ionophore (mg/kg DM)	30.0	30.0	30.0	30.0

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (Eragrostis curvula hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] Contains per kg of premix: 4.06 million international units (MIU) Vitamin A, 30g Monensin sodium (Rumensin 20, Elanco Animal Health), 40.52g Zinc, 7.11g Copper, 1.08g Iodine, 0.2g Cobalt, and 0.3g Selenium (Custom formulation product FE7202,BASF Animal Nutrition, SA)

[3] Spesfeed database.

Table 3.4 Ingredient and chemical composition (%DM) of the four experimental diets (Experiment 2)

	Diets[1]			
Item	WSDRM	EHDRM	CHDRM	MSDRM
Ingredient				
Maize silage				15.0
Eragrostis curvula hay		7.5		
Cottonseed hulls			7.5	
Wheat straw	7.5			
Dry rolled maize	70.0	68.5	73.6	60.1
Molasses	5.0	5.0	5.3	5.0
Cottonseed oilcake	2.5	3.1	2.5	2.5
Wheat bran	11.0	13.0	7.9	14.5
Limestone	1.8	1.8	1.8	1.7
Salt	0.2	0.2	0.2	0.2
Urea	1.0	0.8	1.1	0.9
Trace mineral Premix[2]	0.1	0.1	0.1	0.1
Estimated chemical composition (DM basis) [3]				
DM (%)	86.8	86.7	86.6	70.9
Crude Protein (%DM)	13.3	13.3	13.3	13.3
UIP (%DM)	4.9	4.8	4.9	4.5
DIP(%DM)	8.4	8.3	8.4	8.8
Urea(%DM)	1.0	8.1	1.08	8.5
TDN (%DM)	79.9	80.9	80.9	80.8
ME (MJ/kg)	12.2	12.2	12.3	12.3
NEm (MJ/kg)	8.2	8.2	8.2	8.3
NEg (MJ/kg)	5.6	5.6	5.6	5.6
Roughage (%DM)	7.5	7.5	7.5	7.5
Crude Fibre (%DM)	6.4	6.3	6.5	6.3
NDF (%DM)	20.3	20.0	19.3	20.1
eNDF (%DM)	9.5	8.7	10.0	8.1
Fat (%DM)	3.8	4.0	3.8	3.8
Starch (%DM)	48.6	48.1	50.5	47.1
Ca (%DM)	0.7	0.7	0.7	0.7
P (%DM)	0.4	0.4	0.4	0.5
Salt (%DM)	0.25	0.25	0.25	0.25
Ionophore (mg/kg DM)	30.0	30.0	30.0	30.0

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2] Contains per kg of premix: 4.06 million international units (MIU) Vitamin A, 30g Monensin sodium (Rumensin 20, Elanco Animal Health), 40.52g Zinc, 7.11g Copper, 1.08g Iodine, 0.2g Cobalt, and 0.3g Selenium (Custom formulation product FE7202,BASF Animal Nutrition, SA)

[3] Spesfeed database

3.2.4 Management

Cattle were fed once a day to decrease the variation in daily feed delivery practices, and minimizing potential digestive disorders like acidosis and bloat. This once a day feed allocation is a well established practice in Australian feedlots and in some feedlots in South Africa with the goal to maintain steady intakes, and high DMI and strive to eliminate digestive disorders. Feed delivery commenced at 0600h for the entire 14 day trial period. Clean water was available *ad libitum* for the full duration of the trial. Dry matter intake was recorded during each 14 day period. During this period feed was offered *ad libitum* and the orts weighed back to calculate actual DMI. Animal feed intake is influenced by dietary characteristics, requiring calculation of actual DMI for individual animals per period. Animals were kept in their designated pens for the entire feeding period of 14 days and only handled on the 14th day of each period for sampling of rumen fluid and pH instrument insertion and extraction in the handling facility within 100m from the pens.

3.3 SAMPLING

Samples of rumen fluid were obtained from each animal at established 4 hour intervals on day 13 of every experimental period. A detailed chronology of the experimental sampling procedure is listed in Table 3.5

Table 3.5 Feeding management and the sampling schedule of the experimental animals

Time	Days 1 to 13	Day 14	Day15(day 1 of next Exp.)
1 am			
2 am			
3 am			
4 am			Sampling of rumen fluid
5 am			
6 am	Feeding	Feeding	Feeding of new
7 am		Insertion of pH instrument	experimental diet
8 am		Sampling of rumen fluid	Extraction of
9 am			pH instrument
10 am			from the rumen
11 am			
12 am		Sampling of rumen fluid	
1 pm			
2 pm			
3 pm			
4 pm		Sampling of rumen fluid	
5 pm			
6 pm			
7 pm			
8 pm		Sampling of rumen fluid	
9 pm			
10 pm			
11 pm			
12 pm		Sampling of rumen fluid	

The following rumen fluid sampling procedure where followed: 100ml of rumen fluid was collected using a special rumen fluid sampler tube fixed to a 30ml syringe with a 250 µm mesh at the collection point, supplied by Bar Diamond Inc., Parma, ID, USA. Samples of 20ml were collected through a small orifice in the cannulae from the anterior dorsal, anterior ventral, medial ventral, posterior dorsal and posterior ventral regions within the rumen respectively, pooled and stored on ice (Krause and Combs, 2003). From each pooled sample, a 20ml of rumen fluid filtrate was preserved by the addition of 4ml of 25% H_3PO_4, for the determination of VFA. Preservation of filtrate for NH_3-N was done by taking a 30ml filtrate by the addition of 5ml, 50% H_2SO_4 (De Bruin, 1995). Samples were frozen at -20^oC and stored for later analysis (Beauchemin and Yang, 2005). Filtrate samples of 20ml rumen fluid were also frozen at -20^oC for later analyses of lactate. Four random feed samples were collected from each experimental diet from every consecutive 1 metric ton batch received from the feed mill, pooled and kept at -20^oC for further chemical analysis. All the experimental diets were physically assessed through the Penn State

Forage Particle Separator (PSPS) based on properties of the standard S424 of the American Society of Agricultural Engineers (2001), in four different fractions according to the method described by Kononoff *et al.* (2003) and stored at -20^{o}C for further analysis.

3.3.1 Rumen pH measurements

The reticulo-rumen pH was monitored throughout the experimental periods with an automated indwelling pH meter fitted to a data logger device. The devices were calibrated and tested for both functionality and for pH accuracy using standard pH 4 and pH 7 solutions before each test period commenced (Figure 3.2 and 3.3). Special modification to the canula stoppers made it possible to fix the pH meter device on the inside of the rumen. The insertion and extraction times for the pH instruments in the rumens of experimental animals during the trial periods is tabled in Table 3.5.

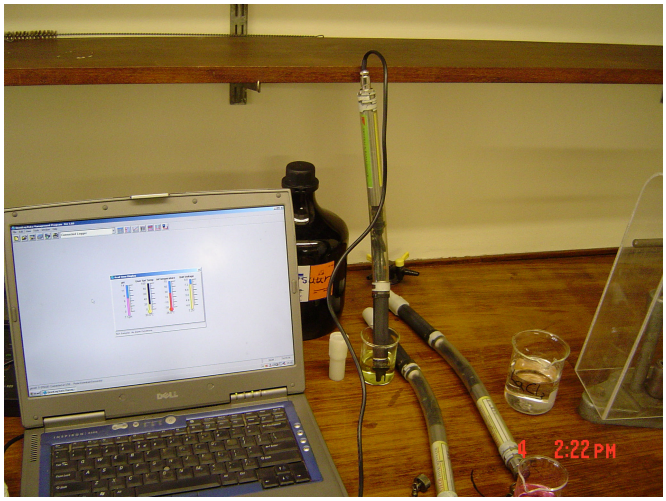

Figure 3.2 Calibration of the instruments in the laboratory with standard solutions for pH 7 and pH 4 to verify the functionality and accuracy of the pH probes

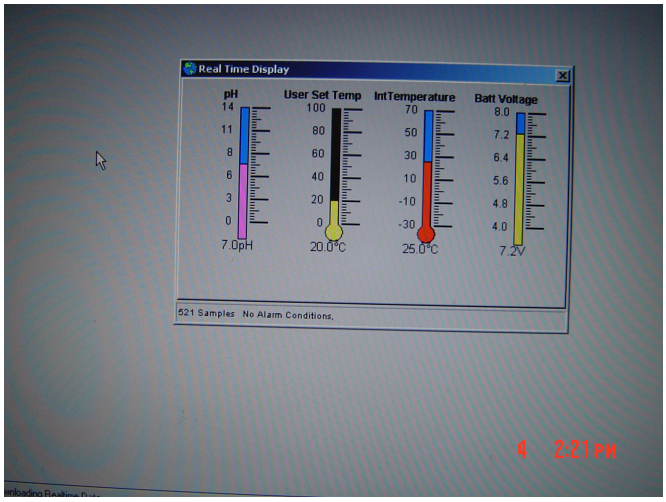

Figure 3.3 The computer software verify the pH values detected from the probe in the standard solution and the instrument can be calibrated by following the calibration steps in the software

3.3.2 The pH meter and data logger

The instrument design is original, self assembled and is functional, easy to use and it is based on documented research by other researchers and scientists. The unique design and functionality of the design warranted the documentation of the following technical note that describes the working, use and design of the instrument in detail for the purpose of this experimental work.

Technical Note: A Method to facilitate continuous Ruminal pH measurement using a standard industrial pH electrode, Data Logger and computer software.

H.J. Vermaak

ABSTRACT

A method is described to construct an indwelling submersible pH electrode and data logger for continuous ruminal pH measurements. The instrument is constructed from 2 major industrial components namely the i) TruTrack Data logger model pH-HR mark 4 supplied by Intech Instruments Ltd; ii) and pH electrode M10 supplied by Innovative Sensors, Inc.; and 2 minor parts consisting of a plastic hose, and stainless steel weight or shroud for the pH probe. Constructing the parts together involves the sealing of the connected pH probe to a data logger with the plastic tube and fitment of the stainless steel weight or shroud around the tip or bulb of the pH electrode. Attachment of the instrument to the cannula stopper is done by a "Key Clip" for ease of placement and retrieval from the cannulated animal. The position of the electrode on the instrument is approximately 400mm inside of the rumen hanging from the cannula stopper, suspended in the middle of the rumen 11 – 15cm dorsally to the ventral sack of the rumen.
Key words: pH, submersible, instrument, probe

The experimental procedure for pH measurement in the rumens of experimental animals is well documented. Methods described ranges from rumenocentesis, using oro-ruminal probes, and spot sampling through a ruminal cannula. Conventional measurement of ruminal fluid pH is a reliable but a very tedious task. Rumenocentesis in studies is limited due to the complicated nature of the surgical procedure and complications in the animal, giving this technique limited scope for field trails (Duffield et al., 2004). Oro-ruminal probes are an alternative and workable probes have been designed and used with great success by Geishauser (1993). Salivary contamination of the spot sample using this method is the greatest concern and might lead to inaccurate pH measurements. Direct ruminal fluid sampling via a rumen cannula is a very common method in research trials (Duffield et al., 2004).This method is also prone to inaccuracy due to exposure of rumen fluid to the aerobic environment. With oxidation of volatile fatty acids, changes in the pH will take place. However the degree of accuracy of this method, spot samples will only reflect the pH at a certain point in time. Continuous measurements using this technique at regular intervals during the day are very labor intensive and might even disrupt animal feeding behaviour.

A continuous indwelling system provides valuable data of post feeding ruminal pH, variation of pH due to type of diet, and variation in animal eating behaviour (Krause and Combs, 2003). Automated indwelling systems have been developed and used in research with success and accuracy (Dado and Allen, 1993; Penner et al., 2006). The majority of early indwelling systems had limitations to the technique as well. External cables linked the indwelling electrode to an external power supply and computer or data logging device to the animal. This concept restricts the mobility of the animal, has potential for mechanical damage to the equipment and is restricted to caged or tethered animals. Stand alone indwelling systems of various designs are currently in use, varying from fixed indwelling electrodes protruding from the external side of the cannula into the rumen, with external cables and external compact data logger and fixed with a leather harness onto the animal, to a compact submersible indwelling electrode and data logger. These submersible pH measuring devices provide an excellent opportunity for the least possible interference with animal feeding, movement behavior and eliminate possible damage to the measuring device from the external environment. The Lethbridge Research Centre pH measurement system (LRCpH), submersible device where tested by Penner et al., (2006) for accuracy and precision and they concluded that it provides accurate, comparable data that can be used for feedlot animals, free stall dairy animals, and grazing studies.

This note presents a technical and practical method for assembly of a submersible pH measuring device using the pH electrodes, compact data logger and computer software from Intech Instruments Ltd. The use of this

instrument applies to any pH research with cannulated large frame bovine species.
The instrument consists of two major components, i) a TruTract Data Logger, Model pH-HR mark 4 (figure 1) supplied by Intech Instruments Ltd. ii) a pH electrode pH model 10 from Innovative Sensors, Inc. (figure 1); and 2 minor components namely a manufactured 210 gram 316 stainless steel weight or shroud with 20mm BSP thread that screw onto the pH electrode (figure 3); and a 250mm 20mm diameter plastic hose.

Figure 1. **TruTrack Data Logger and pH-HR probe used for the assembly of a submersible pH measuring device**

Design:

The TruTract Data Logger, Model pH-HR mark 4 (figure 1) is a small three channel high resolution (16 bit) pH and temperature data logger housed in a rugged 304 stainless steel case. It is 182mm in length and 20mm in diameter and weighs 148g in total. The data logger can be configured to: start on time, immediate start, stop when full, or loop around (overwrite data). Using the OmniLog software, the data logger can be programmed to log pH and temperature through an USB cable before it is placed in the rumen. After completion of target measurements, the data on the logger can be retrieved and stored in a Microsoft windows supported program using the OmniLog software and USB programming cable from Intech Instruments Ltd. The logger connects to an external pH probe through a cable approximately 500mm. These cable connections between the data logger and probe is not water tight. In order to protect the logger, cable and probe from any mechanical and chemical damage, a 20mm diameter PVC, food grade, clear hose can be cut to a length of 250mm for housing of these components. The cable can now be pulled through the hose and the pH electrode can be sheathed by the pipe and clamped to make it water tight. The next step is to attach the cable connector to the data logger's end, and gently coils the length of excess cable in the hose. The data logger and excess cable can now be gently squeezed into the hose so that the hose sheathed the logger approximately 150mm. The hose can be secured to the data logger with hose clamps to get a water tight seal. With this done, the data logger and probe is secure and watertight as well as flexible to withstand normal rumen motility (figure 2).

Figure 2. **The assembled submersible pH measuring instrument is ready for use.**

The stainless steel weight or shroud (figure 3 & 4) is designed to enclose the wet bulb partially to prevent direct contact with the rumen epithelium and eliminate mechanical damage during handling and also to act as ballast to keep the probe in position within the rumen. The weight is designed to be easily detached from the probe by unscrewing from the electrode. This feature is necessary for cleaning and storage of the pH electrode. Shroud dimensions measure 35mm in length, 40mm in diameter, and inside diameter of 30mm and 20mm BSP tapped thread end length of 15mm. Configuration of the enclosure allows rumen fluid to flow or percolate evenly around the electrode during normal rumen motility. Instances where very course fiber diets are being tested, fine sieve material with a 2mm aperture tightly covered and secured around the weight, might prevent course fiber particles to clog or damage the bulb of the electrode. Compaction of the electrode bulb will also inhibit the percolating affect of rumen fluid over the bulb.

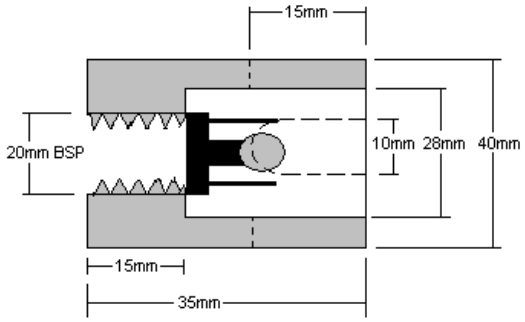

Figure 3. Technical detail drawing of the weight

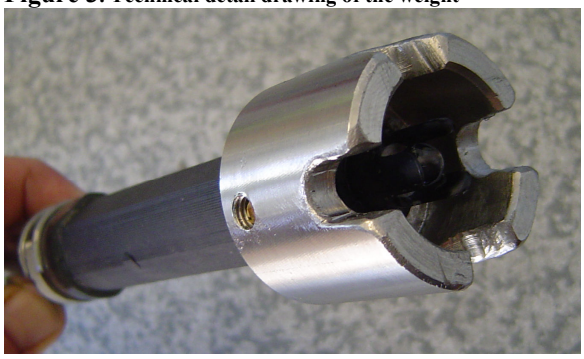

Figure 4. Shows the screw-on stainless steel enclosure configuration

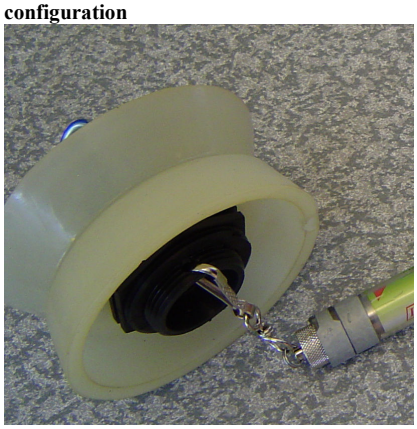

Figure 5. The cannula attachment

The instrument is ready for use and can now be attached to the cannula stopper with an easy removable stainless steel "key clip" (figure 5). The end cap of the data logger is designed for attaching a key ring clip and the water tight end cap will also protect the USB port connector plug on the data logger from any moisture from the rumen environment. Take care to tighten this end cap before placement in the rumen. The total length of the assembled instrument is around 400mm, depending on the length of the key clip. The instrument can now be lowered into the rumen via the cannula after the logger has been programmed (figure 6). Take care that the instrument is tightly secured to the stopper and that the weight is situated above the ventral sack of the rumen. In most cases the position of the probe would be around 10 to 15cm dorsally to the ventral sack in the middle of the rumen.

Figure 6. The placement of the instrument in the rumen

The fundamental design parallel the design of the Lethbridge Research Centre pH measuring system tested by Penner et al., (2006) in many ways although the instrumentation might differ in size and the way data is stored between the different instrument suppliers. Calibration of the instruments pH probe is essential at pH 4 and pH 7 with standard solutions before use. This will compensate or detect any drift or malfunction in sensitivity of the probes before measurements with the instrument commence. Proper cleaning and storage of the electrode's wet bulb will ensure long term use of the instrument.

INSTRUMENT VALIDATION

This indwelling submersible pH electrode and data logger was validated for accuracy and detection of pH as well as drift in pH values over time. The instrument was validated for periods of 24 hours measuring pH readings of standard solutions at pH 4 and pH 7 respectively. No

indication of any drift was detected across the 24h period with 1 minute interval readings and in both instances and pH readings stayed stable at pH 4 and 7 respectively.

ACKNOWLEDGEMENTS

I gratefully acknowledge Dr. David Hutcheson, Animal-Agricultural Consulting, Inc., USA, for his ideas and contribution to the development and special thanks to Andy Weber from Intech Instruments Ltd, New Zealand, for his technical support, assistance and selection of products needed to assemble the submersible pH measuring instrument.

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3.4 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), ether extract (EE)(procedure 920.39 AOAC, 2000), crude protein (CP) was analyzed using Leco analysis (procedure 968.06 AOAC, 2000), neutral detergent fiber (NDF) (Robertson & Van Soest, 1981), digestible NDF (dNDF) (Engels, E.A.N. & Van Der Merwe, F.J., 1967; Tilley, J.M.A. & Terry, R.A., 1963), acid detergent fiber (ADF)(Goering & Van Soest, 1988), starch (MacRae & Armstrong, 1968; Faichney & White, 1983; AOAC, 1990), ash (procedure 942.05 AOAC, 2000), calcium (Ca)(Giron, 1973) and phosphorous (P)(procedure 965.17 AOAC, 2000). Each sieved sample obtained from the Penn State Forage Particle Separator sieves (*Nasco*, Fort Atkinson, WI, USA, Model C15924N) were also analyzed for DM, NDF, ADF, Ca, P and starch.

Volatile fatty acids from rumen fluid were analyzed using gas chromatography (Webb, 1994). Rumen ammonia nitrogen (NH_3-N) from rumen fluid samples was analyzed using the procedure from Broderick & Kang, (1980). Lactate from rumen fluid samples was analyzed using the procedure from Pryce (1969) and Leland Clark, Ohio with sensor technology of the YSI 2300 STAT PLUS Glucose and L-Lactate analyzer (YSI Inc., Yellow Springs, Ohio, USA).

3.5 STATISTICAL ANALYSIS

All the data will be subjected to the Proc GLM model (Statistical Analysis Systems, 2006) analysis which is appropriate for mixed effects models in a Latin Square Design. The statistical state for 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 of interest measured, 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 term. In the model, the animal will be specified as being random and periods will be specified as repeated measurements. Also, the covariance structure will be identified as the compound symmetry assumption. Means and standard errors (SE) were calculated. Significance of difference ($P<0.05$) between means was determined by the Fisher test (Samuels, 1989).

CHAPTER 4.

RESULTS AND DISCUSSION: EXPERIMENT 1

4.1 EXPERIMENTAL DIETS

4.1.1 Chemical composition of experimental diets

Pooled samples of the 4 experimental diets used in the experiment were analyzed and the chemical composition of each treatment diet is shown in Table 4.1

Table 4.1 The chemical composition of the four experimental diets fed to the feedlot animals

	Experimental diets[1]			
Composition (%DM)	WSHC	EHHC	CHHC	MSHC
Dry matter	85.97	86.82	89.04	69.58
Crude Protein	10.65	11.13	11.27	12.21
NFC[2]	48.43	49.53	50.77	46.39
Acid detergent fiber	12.75	9.84	12.29	12.86
Neutral detergent fiber	31.47	29.54	27.30	30.84
Digestible neutral detergent fiber	12.54	15.08	13.39	11.68
Ether extract	5.01	5.11	6.12	5.35
Starch	31.18	30.25	32.78	31.76
Ash	4.44	4.69	4.54	5.21
Calcium	0.57	0.51	0.61	0.68
Phosphorus	0.41	0.45	0.44	0.47

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] Non-fiber carbohydrates calculated as 100- (Crude protein + ash + neutral detergent fibre + ether extract)

The DM levels were comparable with the database formulated values as presented in Table 3.3 across all treatment diets. The crude protein (CP) values were numerically lower than the expected CP values for all treatment diets. The reason for this may be due to variation in consistency of the hominy chop as well as variation in protein content from other feed ingredients such as cottonseed oilcake and wheat bran. Ether extract (EE) values were also slightly lower than expected and this also reflects the variation in consistency of the hominy chop used (C.T Van Der Merwe, personal communication 2008). The ADF value for the *Eragrostis curvula* hay diet were numerically lower than expected but can be attributed to the quality of hay. The NDF values compare well with the expected values, and starch values were also numerically higher than expected but may be due to the inconsistency of starch levels of hominy chop. Sampling errors, mixing inaccuracy and differences between theoretical values used for raw materials in

the formulation program may also have contributed to the differences in chemical composition between the estimated and actual values of the rations.

4.1.2 The composition and particle size distribution of the sieved fractions of the experimental diets as determined by using the Penn State Forage Particle Separator.

Experimental diets were all assessed by sieving method using the Penn State Forage Particle Separator (PSPS) (*Nasco*, Fort Atkinson, WI, USA, Model C15924N). Pooled samples were analyzed for particle size distribution (Table 4.2), and each sample obtained where chemically analyzed for the three different sieve sizes. The chemical composition of the samples for the upper sieve; middle sieve; lower sieve and bottom pan is presented in Table 4.2. All four experimental diets were very similar in their chemical composition with the only difference being the physical assessment of the diets through the PSPS. The WSHC and EHHC diets both contained 2% particles on the sieve with an aperture bigger than 19.5mm compared to none from the CHHC and MSHC diets. This physical difference in particle size might have stimulated more salivation and rumination and this was also reported by Moore *et al.* (1987) that wheat straw diets resulted in longer rumination times compared to alfalfa and cotton seed hull diets. Physical difference in particle size alone may not always fully explain the chewing behaviour and subsequent buffering capacity of feeds and roughages. The peNDF system assumes that forage particle size explains all the variation in chewing response but this is not always true. Mertens (1997) and others suggested that differences in forage fragility, stem brittleness and lignin contents may explain variation in chewing response more than particle size alone. Summarised data from Mertens (1997) for different forages revealed some forages like oat straw may stimulate chewing twice as much as alfalfa hay with similar particle size. Grant (2010) defined fragility as the relative rate at which forage is reduced in particle size during chewing or some laboratory simulation of chewing action while Ulyatt (1983) suggested that "brittleness" of forages and increased particle reduction during eating and rumination may be due to increased lignification of cell walls as forages mature. Fragility may be related to lignin content (Jung and Allen, 1995) and digestibility as well as to anatomical differences among plant species such as wall thickness (Van Soest, 1994). In preliminary research done by Cotanch *et al.* (2007) at Miner Institute, they were able to determine physical effective factor (pef) values for forages through the ball milling method. The change in fragility or pef values versus 24h in vitro NDF digestibility revealed strong R^2 values that explain about 60% of variation in forage fragility. This implicates that forages with high dNDF values have high fragility and forages with low dNDF values have low fragility values.

Table 4.2 The particle size distribution of the four experimental diets differing in roughage source

	Experimental diets[1]			
Particles retained (% DM)	WSHC	EHHC	CHHC	MSHC
Upper sieve (19,5mm)	2.00	2.00	0	0
Dry matter	90.62	91.10	0	0
Acid detergent fibre	34.16	37.92	0	0
Neutral detergent fibre	63.07	70.15	0	0
Digestible neutral detergent fibre	40.27	49.73	0	0
Starch	11.45	6.04	0	0
Calcium	0.25	0.23	0	0
Phosphorus	0.20	0.14	0	0
Middle sieve (7,87mm)	9.00	5.00	14.00	21.00
Dry matter	89.69	90.00	89.90	57.63
Acid detergent fibre	20.75	14.21	27.03	16.76
Neutral detergent fibre	43.61	29.57	38.09	32.48
Digestible neutral detergent fibre	26.14	16.32	30.92	13.12
Starch	19.42	21.33	22.64	27.11
Calcium	0.44	0.59	0.39	0.49
Phosphorus	0.33	0.35	0.35	0.33
Lower sieve (3,175mm)	70.00	62.00	57.00	57.00
Dry matter	89.15	89.20	88.53	75.85
Acid detergent fibre	11.60	10.25	11.36	11.43
Neutral detergent fibre	31.38	29.24	30.83	29.78
Digestible neutral detergent fibre	14.06	13.64	21.27	17.31
Starch	30.11	30.10	28.85	34.19
Calcium	0.61	0.72	0.71	0.69
Phosphorus	0.47	0.45	0.47	0.48
Bottom pan (<3.175mm)	19.00	31.00	29.00	22.00
Dry matter	88.73	87.06	87.56	79.27
Acid detergent fibre	7.05	6.76	6.21	6.49
Neutral detergent fibre	21.37	17.32	18.71	18.86
Digestible neutral detergent fibre	3.91	4.95	4.43	5.50
Starch	44.57	43.72	45.40	48.39
Calcium	0.63	0.78	0.55	0.96
Phosphorus	0.47	0.44	0.47	0.49
Mean particle size (mm)	3.21(SD[2] ± 2.23)	2.60(SD[2]± 2.35)	2.83(SD[2]± 2.41)	3.42(SD[2]± 2.41)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] The standard deviation from the mean.

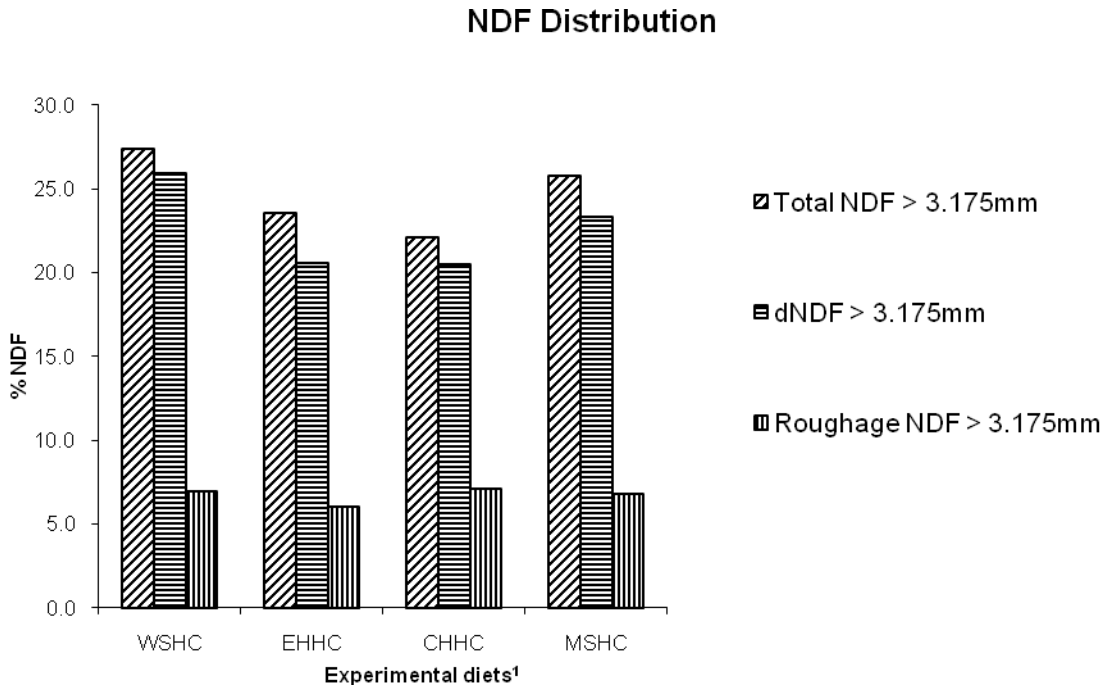
In Table 4.2, percentage of small particle size measured on the bottom pan of the Penn State Forage Particle Separator indicates 31% and 29% for EHHC and CHHC diets respectively, compared to

19% and 22% for WSHC and MSHC diets. However, the partitioning for NDF above the 3.175mm sieve of the PSPS presented in Table 4.3 and Figure 4.1 reveals WSHC diet had 27.4% total dietary NDF above 3.175mm compared to 23.6%; 22.1%; and 25.78% for EHHC; CHHC; and MSHC diets respectively. Total digestible NDF expressed as a percentage of total NDF was also higher for WSHC than all other diets. This observation was partially masked by entrapped NDF from hominy chop and /or wheat bran source with high dNDF. The large pool which includes the contents of the upper sieve and middle sieve portions of the PSPS represented mostly course particles from roughage source. Total dNDF expressed as a percentage of the total NDF for the upper sieve fractions (Table 4.2) are 63.8% for WSHC and 70% for EHHC diets while the middle sieve fractions (Table 4.2) was 74%, 55%, 81% and 40% dNDF as percentage of total NDF for WSHC, EHHC, CHHC and MSHC respectively. Thus, cottonseed hulls represent the highest dNDF values for the large pool size compared to the other roughage sources. In the current study, particle size distribution between diets was very similar in terms of NDF fractions on the PSPS. However, dNDF for cottonseed hulls used in this experiment were higher than the other diets, especially for the large pool fractions (upper and middle sieve fractions) when dNDF was expressed as a percentage of total NDF. This implicates that although all diets were very similar in total NDF and theoretical eNDF values, the cottonseed hull diet had a much lower fragility value and may have resulted in faster particle reduction that elicit less rumination and salivation in the animals compared to the other three diets. In contrast, the dNDF fraction for the MSHC diet for the large forage particle pool (upper and middle sieves on PSPS) in this study also indicated possibly more chewing and rumination needed for particle size reduction to escape from the reticulorumen. With reference to forage particle size, Shain *et al.* (1999) reported no difference in animal performance for different particle size within roughage, indicating forage particle size of the roughage *per se* did not have any influence on growth and carcass performance parameters.

Table 4.3 The effect of roughage source on distribution of NDF fractions above 3.175mm of the four different experimental diets expressed on dry matter basis

	Treatments[1]			
Composition (%DM)	WSHC	EHHC	CHHC	MSHC
Total neutral detergent fiber >3.175mm	27.40	23.60	22.10	25.78
Total digestible neutral detergent fiber >3.175mm	25.92	20.60	20.53	23.31
Roughage neutral detergent fiber >3.175mm	6.98	6.05	7.13	6.82

[1] Experimental diets: WSHC (wheat straw, hominy chop); CHHC (cottonseed hulls, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); MSHC (maize silage, hominy chop)



[1] Experimental diets: WSHC (wheat straw, hominy chop); CHHC (cottonseed hulls, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); MSHC (maize silage, hominy chop)

Figure 4.1 The effects of roughage source on distribution of NDF fractions above 3.175mm for the four different treatments

4.2 THE EFFECT OF ROUGHAGE SOURCE ON VOLATILE FATTY ACID COMPOSITION

4.2.1 Total volatile fatty acid concentration and composition

Total VFA's and molar proportions of the three major VFA's produced in the experimental animals fed different sources of roughage in combination with hominy chop, are summarized in Table 4.8. In this study the average total VFA concentration over 24 hour period ranged between 90 to 122 mM/l with MSHC diet producing lower total VFA concentration ($P<0.05$). These values compares well with a similar study, using similar diets, done by Shain *et al.* (1999) with different forage sources and particle sizes. These researchers reported total VFA values of 116mM/l for Lucerne hay diets with a mean particle size of 2.54cm and 110mM/l for wheat straw diets with a mean particle size of 2.54mm. Peak concentrations of total VFA's were at 6 and 10 hours post feeding for MSHC and CHHC diets respectively, and 14 hours

post feeding for EHHC and WSHC diets (Figure 4.2). Peak VFA concentration coincides with the times of lowest ruminal pH recordings. Total rumen VFA concentration for the experimental animals fed the MSHC diet was the lowest at 90.85 mM/l ($P<0.05$) for the 24 hour period. This was probably due to the fact that maize silage contained a large portion of VFA's produced during the ensiling process and was rapidly transported across the rumen wall. It is quite an acceptable explanation that VFA losses occurred during the feed manufacturing and feeding process through evaporation. This also explains the 4 hour earlier peak production of total VFA's for MSHC diet compared to the other diets (Figure 4.2). However, the composition and volumes of VFA in the maize silage was not measured during this study and the actual contribution of VFA from maize silage can't be reported. Kung *et al*. (1992) also reported a lower but not significant total VFA concentration for cattle fed a maize silage diet in combination with maize grain compared to Lucerne hay in combination with maize grain diets. Acetate: propionate ratios are shown in Table 4.4 and the lowest ratio of 1.41 ($P<0.05$) was for the diet containing cottonseed hulls when compared to the other diets. This observation supports the observations of Khafipour *et al*. (2009) where they induced sub clinical acidosis to cows with grain based diets. The elevation in propionate concentrations and reduction in acetate levels led to an A:P ratio of 1.61 compared to 2.86 for the control diets in their study.

Table 4.4 Mean molar proportions and total volatile fatty acids concentrations produced by steers fed different roughage sources in combination with hominy chop

	Experimental Diets[1]				
	WSHC	EHHC	CHHC	MSHC	SEM[3]
Total VFA production (mM/l)	111.82^{a}	122.15^{a}	115.65^{a}	90.85^{b}	4.15
Acetate (mM/l)	66.04^{ab}	67.93^{a}	58.72^{b}	54.02^{bc}	2.50
Propionate (mM/l)	31.11^{ac}	35.00^{a}	44.83^{b}	23.98^{c}	2.54
Butyrate (mM/l)	12.71^{a}	17.08^{b}	9.38^{a}	11.11^{a}	1.29
A: P Ratio[2]	2.21^{a}	2.05^{a}	1.41^{b}	2.38^{a}	0.18
Acetate (%)	59.1^{a}	55.6^{ab}	50.8^{b}	59.5^{a}	1.82
Propionate (%)	27.8^{a}	28.7^{a}	38.8^{b}	26.4^{a}	1.56
Butyrate (%)	11.4^{a}	14.0^{b}	8.1^{c}	12.2^{ab}	0.70

^{abc} Means in the same row with different superscripts differ ($P< 0.05$)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] Acetate: Propionate ratio

[3] SEM = standard error of the mean

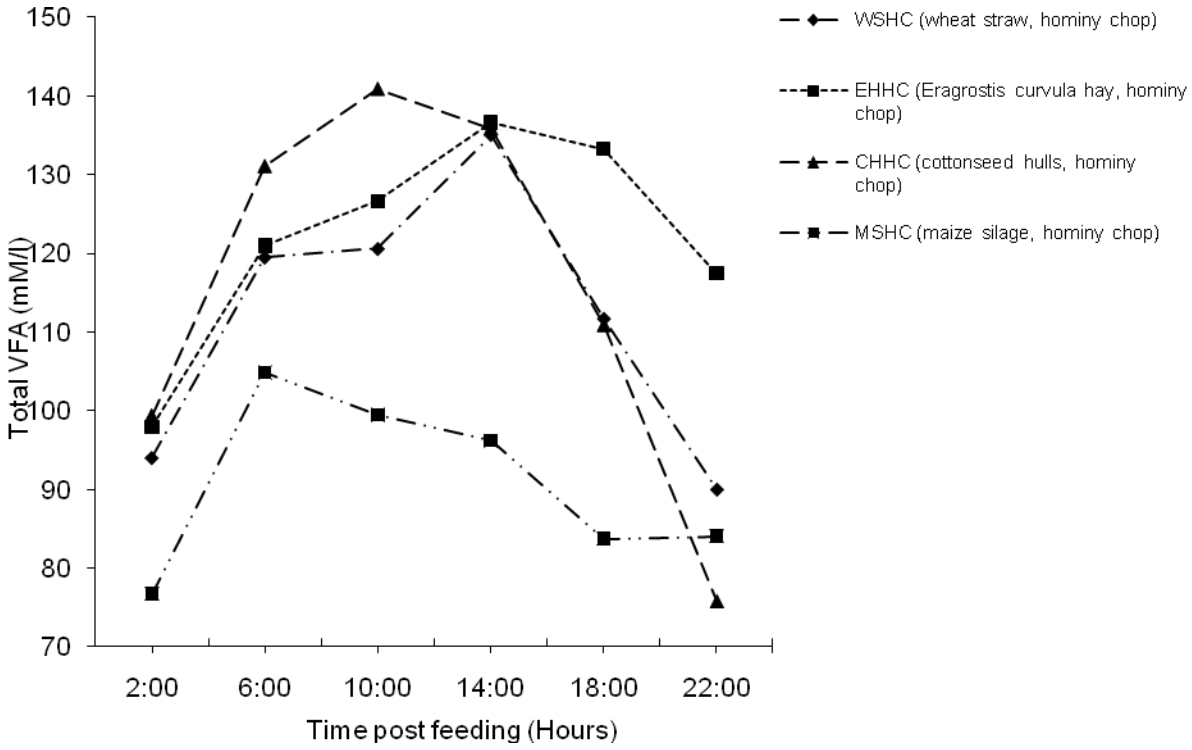


Figure 4.2 Effect of different roughage sources in combination with hominy chop on total volatile fatty acid concentrations produced in the rumen at different time intervals post feeding

4.2.2 Ruminal acetate concentration

Ruminal acetate concentrations are listed in Table 4.5 and illustrated in Figure 4.3. There were no differences observed over the six sampling period mean concentrations for diets containing wheat straw, *Erargostis curvula* hay, and cottonseed hulls as forages. Steers fed the MSHC diet produced the lowest ruminal fluid acetate concentrations ($P<0.05$) when compared to animals fed the EHHC diet, but did not differ from the diets containing the cottonseed hulls and wheat straw. This was probably as result of high acetate levels absorbed directly from the maize silage entering the rumen or loss in acetate through evaporation prior to feeding. This can be seen in the 4 hours post feeding peak concentrations of acetate for animals consuming the MSHC diet. Molar acetate as % of total VFA for the MSHC diet compares well to results found by Soita *et al.,* (2003) with diets containing 80 % concentrate with 20% long cut barley silage. The general depression in acetate is associated with low ruminal pH which is detrimental on fibre digesting bacteria; however, there is a significant interaction between particle size and concentrate level for acetate and this reflects the importance of longer forage particle size when feeding high concentrate diets (Soita *et al.,* 2003).

Table 4.5 The molar concentrations of acetate over time produced by steers fed diets with different roughage sources in combination with hominy chop

	Experimental diets[1]				
	WSHC	EHHC	CHHC	MSHC	SEM[2]
Time	mM/l	mM/l	mM/l	mM/l	
2:00	57.21^{a}	55.99^{a}	52.84^{ab}	46.34^{b}	2.56
6:00	69.64	66.47	66.17	60.80	4.81
10:00	69.86^{a}	69.97^{a}	69.21^{a}	57.43^{b}	1.93
14:00	77.45^{a}	74.33^{a}	66.62^{ab}	55.92^{b}	4.25
18:00	65.78^{abc}	73.79^{a}	55.73^{bc}	50.36^{c}	5.26
22:00	56.34^{ac}	67.04^{a}	41.76^{bc}	53.30^{c}	3.47
Mean	66.05^{a}	67.93^{a}	58.72^{abc}	54.02^{b}	2.50

^{abc} Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cotton hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] SEM = standard error of the mean

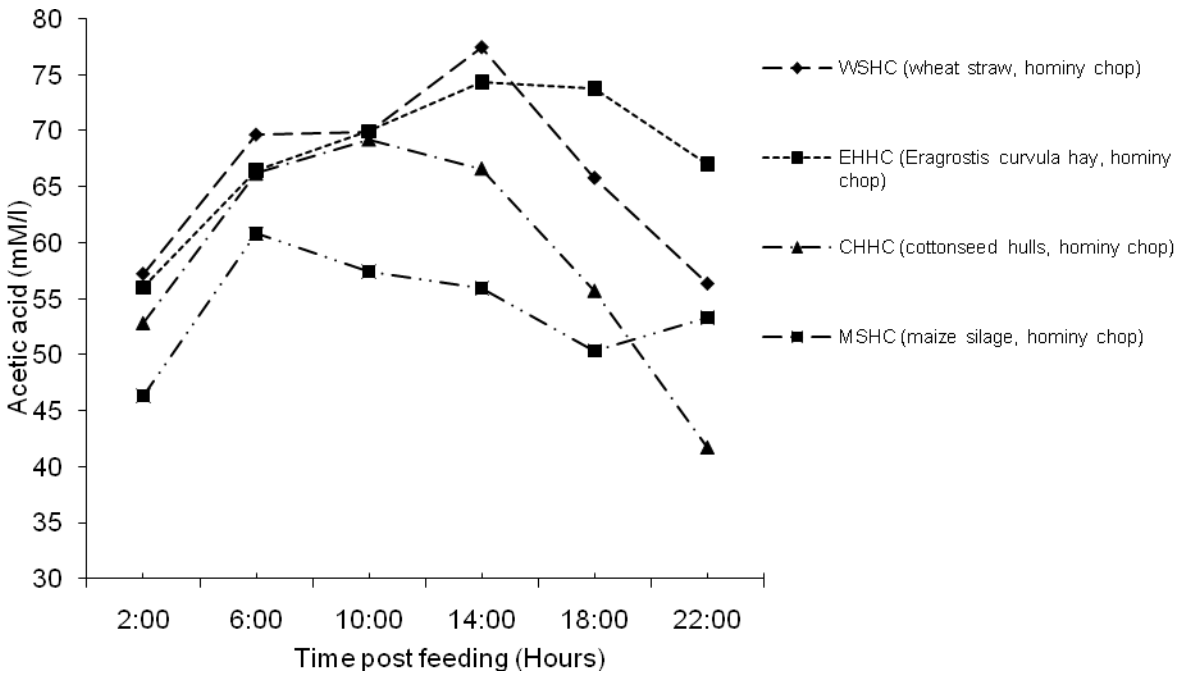


Figure 4.3 The effect of different roughage sources in combination with hominy chop on molar concentrations of acetate produced in the rumen at different time intervals post feeding

4.2.3 Ruminal propionate concentrations

Table 4.6 summarizes propionate concentrations for six different sampling periods. The propionate concentrations were the highest during the first 14 hours post feeding for animals fed the diet containing cottonseed hulls. The cottonseed hull diet also averaged the highest concentration of propionate production namely 44.83 mM/l ($P<0.05$) and differed from the other experimental diets. Molar proportions of propionate produced by animals fed the cottonseed hull diet expressed as a percentage of total VFA was the highest at 38.8% ($P<0.05$) compared to the other experimental diets (Table 4.4). In this trial the cottonseed hull diet consistently produced much more propionate throughout the 24 hour observation period compared to the other diets (Figure 4.4).

Table 4.6 The molar concentrations of propionate over time produced by steers fed diets with different roughage sources in combination with hominy chop

	Experimental diets[1]				
	WSHC	EHHC	CHHC	MSHC	SEM[2]
Time	mM/l	mM/l	mM/l	mM/l	
2:00	24.27^{a}	25.17^{a}	35.91^{b}	19.16^{a}	1.93
6:00	34.33^{a}	33.30^{a}	50.94^{b}	28.51^{a}	3.96
10:00	34.35^{a}	36.81^{a}	57.17^{b}	28.15^{a}	3.76
14:00	40.14^{a}	41.26^{a}	55.41^{b}	26.90^{c}	3.59
18:00	31.07^{a}	40.39^{b}	43.52^{b}	21.58^{c}	2.09
22:00	22.52^{a}	33.06^{b}	26.03^{ab}	19.56^{a}	2.94
Mean	31.11^{ac}	35.00^{a}	44.83^{b}	23.98^{c}	2.54

^{abcd} Means in the same row with different superscripts differ ($P< 0.05$)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] SEM = standard error of the mean

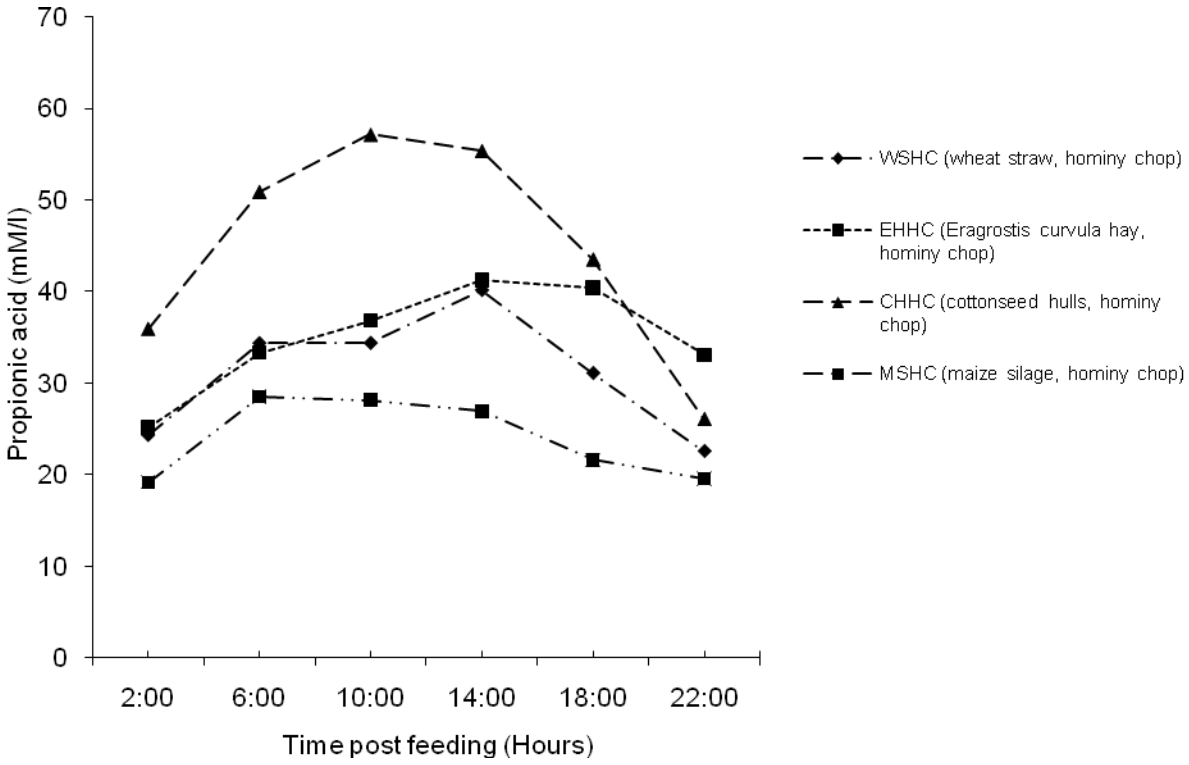


Figure 4.4 Effect of different roughage sources in combination with hominy chop on molar concentrations of propionate produced in the rumen at different time intervals post feeding

4.2.4 Ruminal buterate concentration

Butyrate concentrations in rumen fluid are listed in Table 4.7 and illustrated in Figure 4.5. No difference in mean concentration over the 24 hour measuring period were observed between diets containing wheat straw, cottonseed hulls, and maize silage as roughage source. Animals fed the diet containing *Eragrostis curvula* hay had the highest mean butyrate concentration of 17.08 mM/l over the sampling period and differed from the other diets ($P<0.05$). Butyrate expressed as percentage of total VFA was also higher at 14% ($P<0.05$) for the *Eragrostis curvula* hay diet compared to cottonseed hulls and wheat straw containing diets (Table 4.7). This could be explained since the fractional absorption rate of acetic acid remains relatively unchanged with lowered pH levels but fractional absorption rates increases for propionate and butyrate as pH decreased (Allen, 1997).

Table 4.7 Molar concentrations of butyrate over time produced by steers fed diets with different roughage source in combination with hominy chop

	Experimental diets[1]				
	WSHC	EHHC	CHHC	MSHC	SEM[2]
Time	mM/l	mM/l	mM/l	mM/l	
2:00	10.71^{abc}	14.87^{a}	8.38^{bc}	9.64^{c}	1.42
6:00	13.57^{ab}	19.15^{a}	10.98^{b}	13.43^{ab}	2.18
10:00	14.37^{ab}	17.68^{a}	11.34^{b}	12.02^{b}	1.32
14:00	15.22^{ab}	18.68^{a}	10.68^{b}	11.67^{b}	1.47
18:00	12.86^{ab}	16.92^{a}	8.86^{b}	10.27^{b}	1.48
22:00	9.56^{a}	15.22^{b}	6.03^{a}	9.67^{a}	1.45
Means	12.71^{a}	17.08^{b}	9.38^{a}	11.11^{a}	1.29

[abc] Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cotton hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] SEM = standard error of the mean

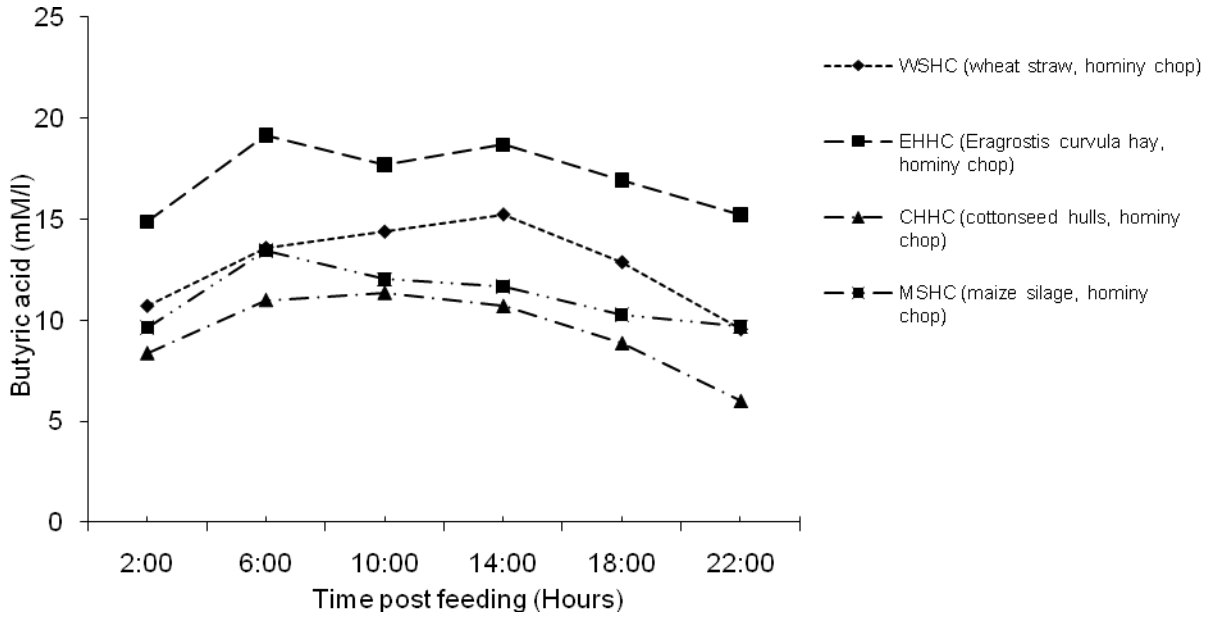


Figure 4.5 The effect of different roughage source in combination with hominy chop on molar concentrations of butyrate produced in the rumen at different time intervals post feeding

4.2.5 General discussion on volatile fatty acid concentrations

The peak VFA concentrations for steers fed the MSHC diet was lower at 90.85 ($P<0.05$) compared to the other diets, and peaked 4 hours before all the other diets. This observation could be explained by the inherent differences in the composition of the MSHC diet itself. Although all four diets were theoretically formulated to contain equal energy, starch, NDF and protein contents, part of the starch content in the MSHC diet was a contribution from the grains contained in the maize silage itself. The MSHC diet only contained 57.9% hominy chop on a dry matter basis compared to 67.3%; 67.9% and 70.7% in the WSHC, EHHC and CHHC diets respectively. This difference implicated that the rate and extent of ruminal starch digestion in the MSHC diet to be different from the other diets. With special reference to maize silage as a roughage source, Firkins (2010) emphasised in his review article that there are not firm estimates for rumen degradable starch in maize silage. Based on a summary of published data on maize silage by Owens (2009), starch digestibility of grains in silage equals that of high moisture maize in the rumen. Starch digestibility from maize silage is more than 94% in the dairy cow and is considered to be more than 97% digestible in the beef steer (Owens, 2009) and this partly explains the 4 hour earlier peak in total VFA production for steers fed the MSHC diet. If starch digestion of the silage grains in the MSHC diet was very high, lower total concentration of VFA could only indicate a possible lesser rumen fermentation of non-forage fibre source since MSHC had lower inclusion of hominy chop (high in NFFS) compared to the other diets. The rate of starch digestibility in the rumen can also be influenced by the interaction when using different sources of maize grain and this was observed in the study by Oba and Allen; (2003) where they fed dry rolled or high moisture maize. The higher rumination and chewing time seems plausible since increased chewing and rumination time have been reported by several researchers (Grant *et al*., 1995; Oba and Allen, 2000; Tjardes *et al*., 2000) evaluating higher NDF silage diets. Tjardes *et al*. (2000) reported that steers fed brown midrib maize silage (BMMS) had higher DMI ($P<0.01$) than steers fed the control silage. This increase in DMI could be partially due to lower NDF concentration and (or) increased NDF digestibility of the BMMS in comparison with the control silage. Grant *et al.* (1995) as well as Oba and Allen (1999) reported higher DMI and faster rumen turnover time with lower NDF and higher dNDF silages compared to higher NDF lower dNDF silages. Tjardes *et al.* (2000) also reported a higher ($P<0.05$) concentration of VFA for BMMS than control. Dry matter intakes for the MSHC diet in the current study was also lower ($P<0.05$) than the other diets which was accompanied by lower concentrations of VFA. The observations and findings of these researchers probably explained more why the MSHC diet had lower VFA concentrations than the theory of ruminal starch digestion alone. Reported ruminal starch digestion in similar studies has not been altered (Oba and Allen, 1999; Grant *et al*., 1995; Tjardes *et al*., 2000). However, none of these researchers evaluated the effects in combination with hominy chop and (or) grinding maize to a very small particle size. Small particle size increases the extent of starch digestion in the rumen and the total digestive tract (Owens, 2009). Most of the starch particles in hominy chop is considered very fine (<1mm) and it can be expected that greater extent of starch digestibility in the rumen

will take place if rumen retention time for these small particles exceeds the rate of digestion. Accompanying high peak VFA concentration and low ruminal pH, the CHHC had lower acetate, and a higher proportion of propionate concentration ($P<0.05$) compared to the other diets. This high propionate concentration in the CHHC diet and a significant shift in A: P ratio as well as rapid depletion of rumen NH_3-N concentrations also indicates a high fermentation rate of starch and NDF entering the rumen. Part of this shift in fermentation might be attributed to the absence of adequate buffering through the process of salivation and rumination which buffers the rumen and prevent a decrease in ruminal pH. The higher fragility of the cottonseed hull diet resulted in higher DM intakes and possibly higher rumen fill and turn over with consequently higher total VFA concentration. Shain *et al.* (1999) compared an all concentrate diet with forage containing diets using Lucerne hay, wheat straw and corn cob diets containing equal amounts of NDF and observed a trend towards higher VFA concentrations and higher rumen DM fill for corn cob and all concentrate diets compared to alfalfa and wheat straw diets. Calsamiglia *et al.* (2008) concluded in their study that the effects of feeding a high-concentrate diet on rumen fermentation are due to a combination of pH and substrate and the digestion of organic matter in high concentrate diets is likely limited by the pH-induced effects on the microbial population activity. This implicates the self-regulating mechanism of the rumen ecosystem against ruminal acidosis. Thus, the supplementation of highly degradable diets result in an increase in organic matter true digestibility (OMTD) and VFA concentration and reduction in pH which in turn reduce OMTD and VFA production in the rumen (Calsamiglia *et al.,* 2008).

4.3 EFFECT OF ROUGHAGE SOURCE ON RUMEN AMMONIA NITROGEN CONCENTRATION

The effects of roughage source on rumen NH_3-N concentration at various time intervals are shown in Table 4.8 and illustrated in Figure 4.6. There were no differences between the different experimental diets ($P>0.05$) and the absence of diet differences is probably because the experimental diets were formulated to have equal concentrations of soluble protein. The profiles for NH_3-N concentrations are in agreement with results found by Calsamiglia *et al*. (2002) and Rotger *et al*. (2005), however, mean concentrations for NH_3-N in this study were lower than reported by those researchers. Rotger *et al*. (2005) used diets containing 151.0 g/kg dietary CP (DM) and roughage: concentrate ratio of 12:88 and 30:70; compared to the average of 11.32 g/kg CP (Table 4.1) and 8:92 roughage: concentrate ratio in this study. The NH_3-N concentrations from this study were comparable with NH_3-N concentrations reported by Goad *et al*. (1998) for grain-adapted animals, and Estell & Galyean (1985) where cattle were fed diets containing 85% concentrate. Initial concentrations of NH_3-N were high at 2 hours post feeding, ranging between 5 and 12 mg NH_3-N/dl. The mean NH_3-N concentrations over 24 hours for all the treatments were in the range of between 2.89 and 5.93 mg NH_3-N/dl. These values were in general lower than the suggested 5mg/dl,

required for maximum microbial protein synthesis (Satter and Slyter, 1975; Kennedy and Doyle, 1992). However, results from the study by Slyter *et al.* (1976) support the view that NH_3-N concentrations of 2 to 5 mg NH_3-N/dl of rumen fluid is sufficient to allow maximum growth of rumen microbes. This statement was also supported by Schaefer *et al.,* (1980) who observed that many predominant species of ruminal bacteria grown in pure cultures could achieve 95% of their maximum specific growth rate in a medium containing less than 2mg NH_3-N/dl. Cellulolytic bacteria mainly require NH_3 as source of nitrogen (Hoover, 1986) and although no rumen digestibility data for cellulose were collected during this experiment, a reduction in cellulose digestion would be expected. This is because rumen fermentation associated with high concentrate diets does not favour cellulose digesting bacteria as result of lower ruminal pH and low concentrations of available NH_3. Concentrations for NH_3-N rapidly decreased to levels as low as 1.44 mg NH_3-N/dl, 6 hours post feeding most probably due to rumen microbial populations utilizing the ammonia.

Ammonia exists as free unionized NH_3 at high pH but exists as ammonia ions (NH_4) at low pH (Erasmus, 1985) and NH_3-N passage across the epithelium mainly occurred by diffusion of the unionized form and some (NH_4) diffusion may be accompanied by the absorption of VFA anions (Remond *et al.,* 1993) Thus, more ammonia is absorbed at high pH than at low pH. Results from the present study indicated pH values that were comparable (generally less than pH 6) to results found by various researchers who fed high concentrate diets to beef cattle (Nagaraja *et al*., 1981; Estell & Galyean, 1985; Goad *et al*., 1998; Cooper *et al*., 1999; Shain *et al*., 1999 and Sindt *et al*., 2004). Reasons for low ruminal NH_3 concentrations in these studies suggest significant amounts of degradable protein available with significant amounts of available high fermentable energy sources (Erasmus, 1985). Of particular importance is the specific growth rate of the microbial population. Furthermore, recycling of nitrogen via salivary urea and its ruminal degradation assures rumen microbes of adequate NH_3 supply (Erfle *et al.,* 1982). The EHHC and WSHC diets had numerically higher concentrations of NH_3 between 6 and 10 hours post feeding but not significantly higher compared to the other diets. This observation may support that higher rumination and salivation with possibly higher recycling of blood urea through saliva was stimulated in the animals by these two diets. This was also confirmed by Soita *et al.,* (2000) when comparing barley silage with different lengths and different rumination patterns. Calsamiglia *et al*. (2002) also reported that constant low pH reduced protein degradation and increased non-ammonia N and dietary N flow compared to higher pH values. This statement may only be applicable to some dietary protein sources degradability in the rumen. To summarize the results found in the current research, rumen ammonia concentration levels did not differ ($P>0.05$) but results indicate a high turnover rate of NH_3-N and possible high microbial protein production in the rumens of animals fed the CHHC diet and the WSHC diet.

Table 4.8 The effect on NH_3-N concentration in the rumen over time produced by steers fed diets with different roughage source in combination with hominy chop

	Experimental diets[1]				
	WSHC	EHHC	CHHC	MSHC	SEM[2]
Time	mg NH_3-N/100ml	mg NH_3-N/100ml	mg NH_3-N/100ml	mg NH_3-N/100ml	
2:00	8.14	9.36	5.24	11.27	2.34
6:00	1.62	8.52	1.95	4.62	2.29
10:00	3.61	6.99	1.95	2.54	2.68
14:00	1.44	4.47	3.51	2.15	0.94
18:00	1.52	3.38	2.39	2.41	0.59
22:00	1.66	2.86	2.27	2.53	0.52
Means	3.00	5.93	2.89	4.25	1.21

[abc] Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] SEM = standard error of the mean

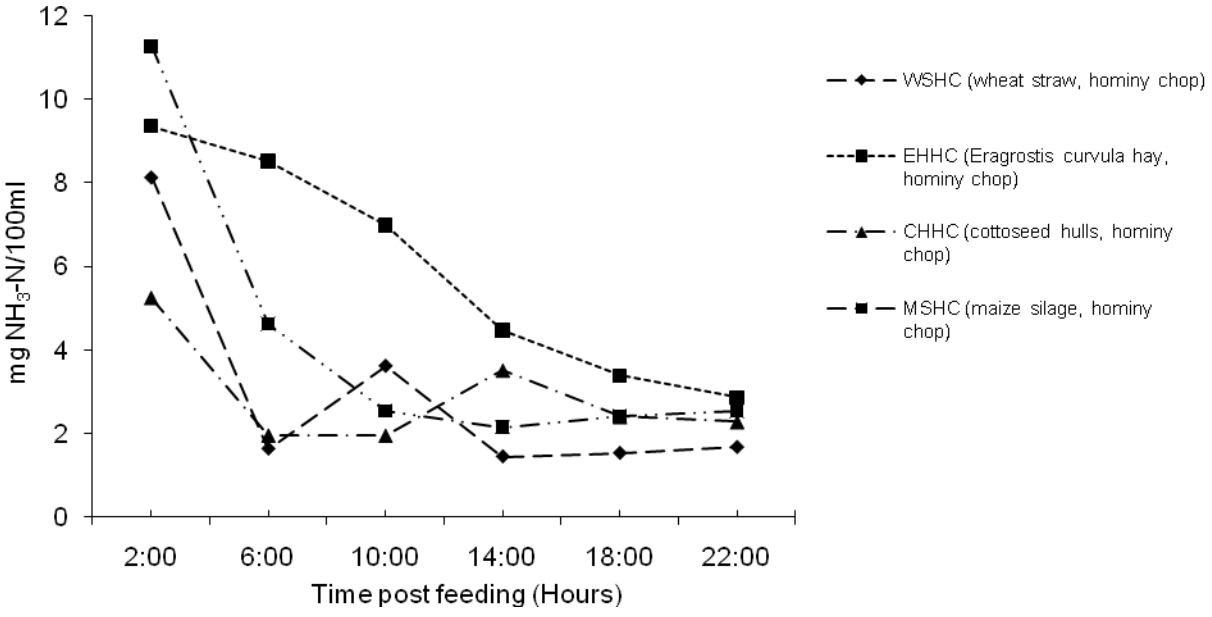


Figure 4.6 The effect of different roughage sources in combination with hominy chop on NH_3-N concentrations produced in the rumen at different time intervals post feeding

4.4 THE EFFECT OF ROUGHAGE SOURCE ON LACTIC ACID CONCENTRATION

Lactic acid concentrations for the different treatments at various time intervals are presented in Table 4.9 and illustrated in Figure 4.7. The mean total lactic acid concentrations did not differ across treatments over the 24 hour collection period ($P>0.05$). However, the lactic acid concentrations in the animals fed the WSHC diet differed ($P<0.05$) from the CHHC and MSHC diets 10 hours post feeding respectively. This is unexplained and questionable since all the other experimental diets followed a decreasing pattern for lactic acid concentrations over time post feeding and this sudden elevated measurement at 10 hours post feeding could be due to errors in laboratory calculation of concentration or inaccuracy in method used to determine the results for these samples. Concentrations in the present study are expressed as mg L (+) lactate per 100ml and equates to concentrations ranging between 0,16mM/l and 0.35mM/l at 2 and 14 hours post feeding respectively. Compared to the results of Burrin & Britton (1986), peak total ruminal lactate concentrations was much lower than the concentration of 1.5mM/l observed 4 hours post-feeding in their study. Khafipour *et al.* (2009) reported levels of 2.29 mM/l where they induced sub acute ruminal acidosis. In contrast to the research done by Khafipour *et al*. (2009), the experimental animals in this study were all well adapted to high energy diets unlike the animals in their study where they induced sub acute ruminal acidosis with a sudden challenge increasing the ruminal starch contents. The times of peak lactic acid production and lowest pH values did not coincide and the contribution of lactic acid as primary drive for low ruminal pH in this study is questionable. These lower concentrations observed can be attributed to measuring L (+) lactate alone without accounting for the D (-) lactate form in the present study as well as a lower ruminal starch load compared to the diets fed by Burrin & Britton (1986). The net absorption rates differ between the 2 isomers and it has been reported that net portal absorption of L (+) lactate is greater than that of D (-) lactate (Harmon *et al*., 1985). Ruminal concentrations of lactic acid are usually not present in large volumes because of utilization by lactate utilizing bacteria and total acid load experienced by an animal may be of more significance during acidosis than lactic acid alone, particularly in sub acute situations (Huntington *et al*., 1980).

Table 4.9 The effect on lactic acid concentrations in the rumen over time produced by steers fed diets with different roughage source in combination with hominy chop

	Treatments[1]				
	WSHC	EHHC	CHHC	MSHC	SEM[2]
Time	mg/100ml	mg/100ml	mg/100ml	mg/100ml	
2:00	2.96	1.88	1.91	3.72	0.65
6:00	2.11	2.17	1.62	1.83	0.35
10:00	3.13[a]	2.01[ab]	1.46[b]	1.56[b]	0.38
14:00	2.19	2.01	1.25	1.66	0.31
18:00	2.21	1.90	1.85	1.96	0.41
22:00	2.39	2.18	1.91	2.04	0.30
Averages	2.41	2.02	1.67	2.13	0.27

[abc] Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] SEM = standard error of the mean

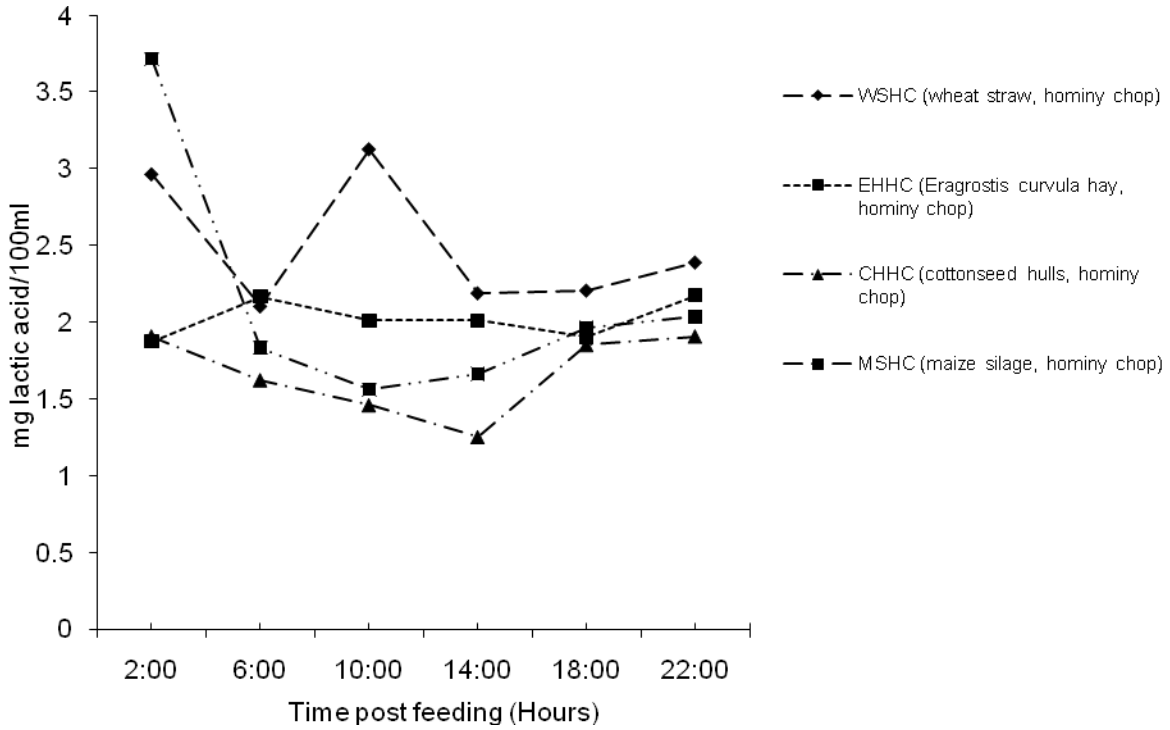


Figure 4.7 The effect of different roughage sources in combination with hominy chop on lactic acid concentrations produced in the rumen at different time intervals post feeding

4.5 THE EFFECT OF EXPERIMENTAL DIETS ON RUMEN pH

The variation in ruminal pH for the four different experimental diets fed to the animals are listed in Table 4.10 and illustrated in Figure 4.8. The pH decreased for the first 10 hours post feeding for all experimental diets.

Table 4.10 The effect of the different roughage sources in combination with hominy chop as energy source on rumen pH at two hour intervals post feeding

	Experimental diets[1]				
Time	WSHC	EHHC	CHHC	MSHC	SEM[2]
0:00	6.45[a]	6.25[a]	5.83[b]	6.35[a]	0.122
2:00	6.13[a]	6.08[a]	5.35[b]	6.05[a]	0.071
4:00	5.95[a]	5.93[a]	5.18[b]	5.80[a]	0.161
6:00	5.73[a]	5.88[a]	5.15[b]	5.63[a]	0.134
8:00	5.90[a]	5.63[ab]	5.20[b]	5.73[ab]	0.158
10:00	5.70[a]	5.58[ab]	5.15[b]	5.68[a]	0.145
12:00	5.50	5.45	5.28	5.68	0.123
14:00	5.63	5.58	5.55	5.75	0.208
16:00	5.85	5.53	5.70	6.00	0.189
18:00	6.03	5.78	5.83	6.20	0.201
20:00	6.18	5.90	5.98	6.38	0.183
22:00	6.20	6.03	5.93	6.53	0.233
Means	5.95[a]	5.80[ab]	5.50[b]	5.98[a]	0.117

[abc] Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] SEM = standard error of the mean

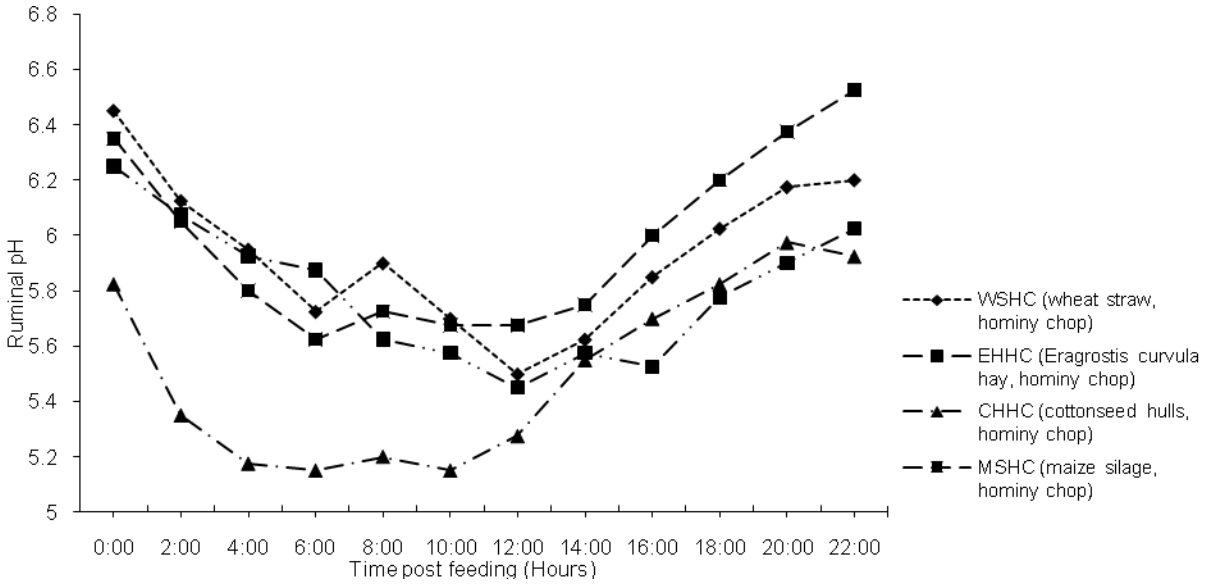


Figure 4.8 Effect of different roughage sources in combination with hominy chop on variation of pH in the rumen at different time intervals post feeding

The pH values observed in this study is in agreement with results reported by other researchers (Moore *et al*., 1987; Shain *et al*., 1999). In the study conducted by Moore *et al*. (1987), rumination time for feedlot finishing consuming diets containing 90% concentrate and 10% wheat straw was higher compared to diets containing 10% Lucerne hay and 10% cottonseed hulls (308 vs. 180 and 210min/d, respectively). The ruminal pH was numerically higher for wheat straw diets compared to diets that contained either Lucerne or cottonseed hulls (pH of 6.2 vs. 5.9 and 5.8, respectively) (Moore *et al*., 1987). This higher ruminal pH was as result of longer rumination time and possibly more stimulated salivation and recycling of bicarbonate to the rumen. Mean pH values in our study did not differ between experimental diets containing wheat straw, *Eragrostis curvula* hay and maize silage as roughage source ($P>0.05$) (Table 4.10). The pH values rapidly decreased for all four treatments until 10 hours post feeding, but after12 -24 hour post feeding pH for all treatments gradually increased to levels above pH 5.9 which coincide with the time when presumably rumination peaked. Animals fed the CHHC diet had the lowest mean pH of 5.5 ($P<0.05$) for the 24 hour observation period. Sub-acute acidosis is generally characterized 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). The response to sub-acute acidosis is lowered feed intakes but even in metabolism studies it is hard to access symptoms (Cooper *et al*., 1998). Considering the two threshold pH values and the total time spent during a 24 hour period below these pH values (Table 4.11 and Figure 4.9), steers fed the CHHC spend a total of 940 minutes below pH 5.6 and differed ($P<0.05$) from animals fed the WSHC; EHHC; and MSHC diets with 257,5; 317,5; and 207,5 minutes respectively. This long period spent below pH 5.6 for CHHC was expected since this diet also had much higher VFA concentrations produced by the experimental animals during the first 14 hours post feeding compared to the other diets. Furthermore, the

production of propionate was also higher (P>0.05) compared to the other diets. None of the experimental animals in this study displayed physical signs of sub-acute acidosis and maintained DMI throughout each period and it is well documented by Allen (1997) that the balance between production of fermentation acids and secretion of salivary buffers is the primary determinant of ruminal pH.

Ruminal pH data from this study showed the lowest mean pH of 5.50 was recorded for steers fed the CHHC diet (P<0.05). These animals fed the CHHC diet also recorded the longest time spent below pH 5.6 and pH 5.2 (P<0.05) respectively. In theory this diet causes concerns for sub-clinical and acute acidosis, but the length of time spent below pH 5.2 for an animal to experience acidosis can't be defined, since it will depend on several factors which included breed, estrogenic implants and meal frequency (Owens *et al*., 1998).Steers fed the WSHC, EHHC and MSHC diets all had very similar profiles with no differences for mean ruminal pH (P>0.05) observed amongst these diets as well as the length of time period pH spent below 5.6. This could be explained by the physical difference in fibre source of wheat straw and *Eragrostis curvula* hay compared to cottonseed hulls. Both the WSHC and EHHC diets might have stimulated more salivation and rumination due to the particle size difference in fibre length which kept ruminal pH more stable without extended periods below pH 5.6. The reason why steers fed the MSHC diet had similar profiles and mean ruminal pH compared to WSHC and the EHHC diets, could be explained by both dilution effects of fermentation acids due to the high moisture content of the MSHC diet and possibly more rumination and salivation as well as reduced ruminal starch digestion which normally elevates rumen VFA production, alters A:P ratio and ultimate drives ruminal pH lower. Similar profiles but non-significant differences between steers fed the experimental diets for the time pH spent below 5.2 (Table 4.11) were observed.

Table 4.11 The effect of different roughage sources on the time periods below pH thresholds 5.6 and 5.2 respectively during a 24 hour period.

	Experimental diets[1]				
	WSHC	EHHC	CHHC	MSHC	SEM[2]
Time	Minutes	Minutes	Minutes	Minutes	
Minutes < pH 5.6	257.5^{a}	317.5^{a}	940^{b}	207.5^{a}	130.82
Minutes <pH 5.2	56.25^{abc}	111.25^{abc}	388.75^{ab}	17.5^{ac}	105.56

^{abc} Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] SEM = standard error of the mean

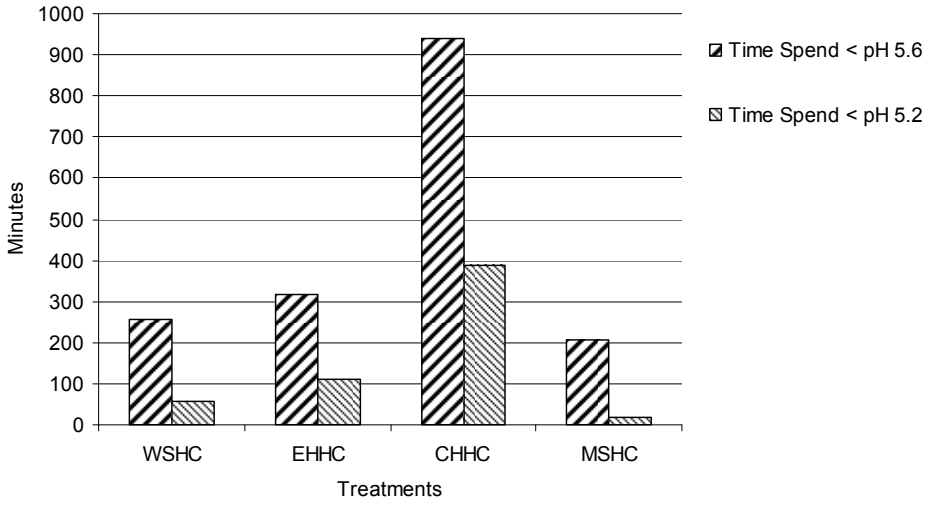


Figure 4.9 The effect of the different roughage sources in combination with hominy chop on the time period spent below pH thresholds 5.6 and 5.2 respectively for the 24 hour period

4.6 DRY MATTER INTAKE

The dry matter intake (DMI) of steers fed the experimental diets is presented in Table 4.12. Animals receiving the diet containing maize silage had a DMI of 147,0 g/kg $LW^{0,75}$ /day ($P<0.05$) and differed from those diets containing cottonseed hulls and *Eragrostis curvula* hay. Dry matter intakes of the steers fed the MSHC were significantly lower than intakes for the other diets and this was also reported by Mader *et al.,* (1991) which compared similar diets to maize silage based diets. Reasons for this may be attributed to physical fill effect in the rumen and longer mean retention time. Soita *et al.* (2003) demonstrated this effect comparing long and short particle silages with high concentrate diets and found higher rumen turnover rates using short particle silages.

Table 4.12 The effect of different roughage sources in combination with hominy chop as energy source on dry matter intake (DMI) of steers

	Experimental diets[1]				
	WSHC	EHHC	CHHC	MSHC	SEM[2]
Mean DMI (g/kg $LW^{0,75}$ /day)	160,0[ab]	170.0[a]	166.0[a]	147.0[b]	7.0

[ab] Means in the same row with different superscripts differ ($P< 0.05$)

[1] Experimental diets: WSHC (wheat straw, hominy chop); EHHC (*Eragrostis curvula* hay, hominy chop); CHHC (cottonseed hulls, hominy chop); MSHC (maize silage, hominy chop)

[2] SEM = standard error of the mean

Observations and results shown significant differences in some rumen parameters like volatile fatty acid production in the rumens of steers fed the experimental diets and these results suggest interactions between roughage source and non structural carbohydrate source. Non-significant differences were observed in mean lactate production, ruminal pH and time period pH were below 5.6. This observation suggests little interaction between roughage source and non structural carbohydrate source on rumination and buffering of the rumen and warrants further investigation. The next experiment was conducted to test if observations found in the current experiment were different using the same roughage sources in combination with dry rolled maize as non structural carbohydrate source.

CHAPTER 5.

RESULTS AND DISCUSSION: EXPERIMENT 2

5.1 EXPERIMENTAL DIETS

5.1.1 The nutrient composition of the experimental diets

Pooled samples of each experimental diet used in the four treatments were analyzed and the chemical composition of each treatment diet is presented in Table 5.1. Despite formulation of diets to achieve equal CP values, the WSDRM and EHDRM actual crude protein values was lower than CHDRM and MSDRM. This may be a result of inconsistent CP values for wheat straw and *Eragrostis curvula hay* as well as by-products like cottonseed oilcake and wheat bran used in these diets. However, crude protein values satisfied the NRC (2000) recommendations for maintenance and growth of 300kg steers with an ADG of 1.5kg for all experimental diets. Subtle differences in nutrient composition between diets were mainly due to sampling errors. The DM levels were comparable to database formulated values across all experimental diets. The NDF values also compared to database formulated values.

Table 5.1 The chemical composition of the four experimental diets fed to feedlot animals.

	Experimental diets[1]			
Composition (%DM)	WSDRM	EHDRM	CHDRM	MSDRM
Dry matter	87.39	88.82	87.75	70.51
Crude Protein	11.51	11.78	12.25	12.65
NFC[2]	62.78	62.48	60.23	61.14
Starch	49.30	50.09	47.11	50.56
Acid detergent fibre	8.01	8.74	9.50	9.15
Neutral detergent fibre	19.76	19.37	20.92	19.47
Digestible neutral detergent fibre	8.98	7.88	8.66	5.26
Ether extract	2.57	2.56	2.98	2.72
Ash	3.38	3.81	3.62	4.02
Calcium	0.49	0.56	0.53	0.64
Phosphorus	0.33	0.31	0.36	0.34

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2] Non-fibre carbohydrates calculated as 100- (Crude protein + ash + neutral detergent fibre + ether extract)

5.1.2 The composition of the sieved fractions of the treatment diets as determined on the Penn State Forage Particle Separator.

Experimental diets where all sieved with the Penn State Forage Particle Separator (PSPS). Pooled samples were analyzed for particle size distribution and each sieved sample obtained where chemically analyzed for the four different particle sizes. The chemical composition of the samples for the upper sieve, middle sieve, lower sieve and bottom pan is presented in Table 5.2. The particle sizes of the four different diets in this experiment did not differ from each other in terms of NDF distribution and were truly balanced to have about 7% of NDF from roughage in all diets. However, as can be seen in experiment 1 with MSHC, MSDRM also had the largest mean particle size of 4.04 mm compared to the other diets with a mean particle size of 3.57 mm; 3.64 mm; and 3.59mm for WSDRM; EHDRM; and CHDRM respectively. This can be explained by the difference in particle size of the maize silage itself, containing a large portion of whole grain kernels. The observed mean particle size of the diets in this experiment was also numerically larger (3.57 mm – 4.04 mm) compared the mean particle size (2.6 mm -3.42 mm) observed in experiment 1. This incremental shift in particle size for these diets was mainly as a result of the larger particle size of the dry rolled maize as opposed to that of hominy chop.

Table 5.2 The particle size distribution of the four experimental diets differing in roughage source

	Experimental diets[1]			
Composition (% DM)	WSDRM	EHDRM	CHDRM	MSDRM
Upper sieve (19.5mm) [2]	0.00	6.00	4.00	0.00
Dry matter	0.00	85.82	91.37	0.00
Starch	0.00	17.07	14.30	0.00
Acid detergent fibre	0.00	14.18	32.62	0.00
Neutral detergent fibre	0.00	29.41	43.48	0.00
Digestible neutral detergent fibre	0.00	17.11	30.70	0.00
Calcium	0.00	0.76	0.62	0.00
Phosphorus	0.00	0.26	0.32	0.00
Middle sieve (7.87mm) [2]	14.00	8.00	11.00	20.00
Dry matter	89.61	90.10	91.11	63.61
Starch	44.93	30.26	23.10	41.85
Acid detergent fibre	9.97	15.73	27.33	14.74
Neutral detergent fibre	21.73	35.14	42.21	34.18
Digestible neutral detergent fibre	11.67	18.00	29.60	9.90
Calcium	0.19	0.11	0.56	0.29
Phosphorus	0.26	0.49	0.31	0.27
Lower sieve (3.175mm) [2]	73.00	71.00	69.00	70.00
Dry matter	88.47	89.76	88.49	74.60
Starch	55.09	56.14	59.80	53.40
Acid detergent fibre	7.29	6.09	5.11	6.06
Neutral detergent fibre	18.50	13.84	13.29	21.43
Digestible neutral detergent fibre	6.67	4.17	3.89	4.98
Calcium	0.41	0.55	0.48	0.70
Phosphorus	0.28	0.21	0.22	0.31
Bottom pan[2]	13.00	15.00	16.00	10.00
Dry matter	89.19	90.67	89.57	68.42
Starch	28.08	33.20	30.72	31.27
Acid detergent fibre	11.08	8.04	7.76	8.33
Neutral detergent fibre	27.90	19.17	22.64	26.82
Digestible neutral detergent fibre	14.92	9.63	10.60	10.55
Calcium	1.14	1.72	1.31	1.84
Phosphorus	0.62	0.46	0.64	0.62
Mean particle size (mm)	3.57(SD[3] ± 2.01)	3.64(SD[3] ± 2.34)	3.59(SD[3]± 2.31)	4.04(SD[3]± 2.04)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2] Particles retained on sieve

[3] Standard deviation

The results from different fractions of NDF found on the PSPS are summarized in Table 5.3 and illustrated in Figure 5.1. Dietary concentration of roughage NDF >3.175mm for the WSDRM was higher than the other diets. The total sum of dNDF for each fraction above 3.175mm was calculated and WSDRM were lowest at 12.44% followed by EHDRM at 12.68% compared to 13.74% and 14.66% for CHDRM and MSDRM respectively. The upper sieve and middle sieve fractions (Table 5.2) of the PSPS represented mostly course particles from the roughage source. Total dNDF expressed as a percentage of the total NDF for the upper sieve fractions on the PSPS (Table 5.2) comprised 58.2% and 70.6% for EHDRM and CHDRM diets respectively. The middle sieve represents 53.7%, 51.2%, 70.1% and 29% dNDF as percentage of total NDF for WSDRM, EHDRM, CHDRM and MSDRM respectively. Thus, cottonseed hulls in this experiment represent high NDF levels but this diet have on average higher dNDF values than the other roughage sources for the upper and middle sieve fractions. In contrast to CHDRM diet, the MSDRM diet had the lowest dNDF for these sieve sizes. The general relationship between NDF digestibility and fragility establish by Grant (2010) suggested that hays with similar particle size and different digestible NDF elicits different chewing behaviour in animals. This means hay with high fragility and high dNDF values elicits less chewing time, more particle reduction and less buffer delivery to the rumen due to salivation. In the current study there were similar trends in terms of fragility and its effect on buffering through chewing and rumination, especially regarding at the large pool size (upper and middle sieves on PSPS) for the four different diets. The CHDRM diet had the highest dNDF value followed by EHDRM and WSDRM and lowest dNDF value for MSDRM.

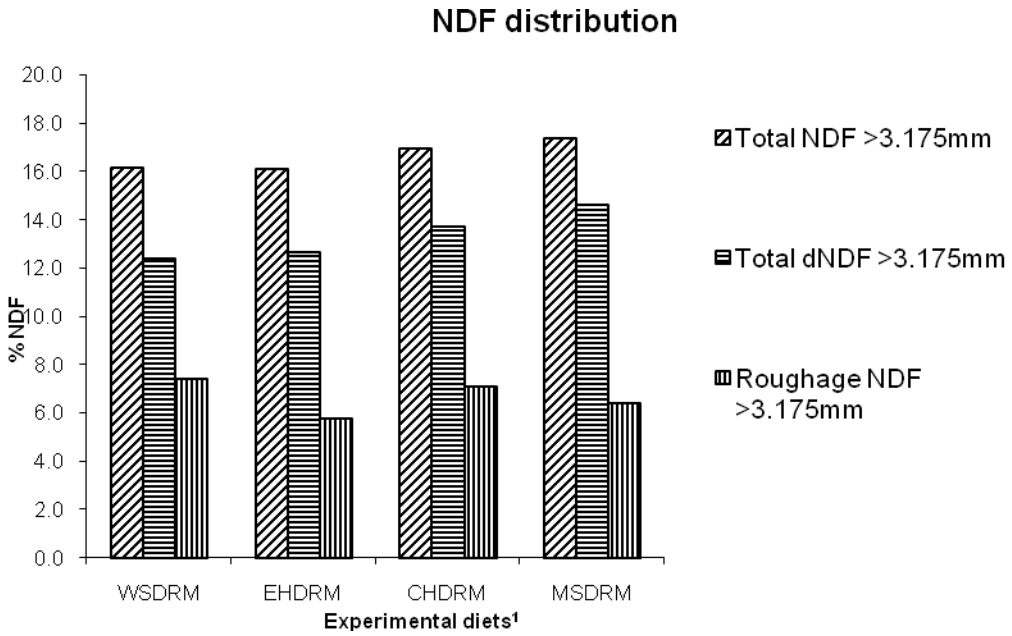
Basalan *et al*. (2002) reported NDF disappearance at 96h for wheat straw in 90% concentrate diets to be higher ($P<0.05$) than alfalfa hay in combination with 90% concentrate. Interestingly the rate of disappearance of hemicelluloses and ADF, expressed as a fraction of that disappearing at 96h were quadratically related to pH with disappearance being minimum at pH5.9 and 5.4 respectively (Basalan *et al.*, 2002). Possibly, this can be contributed to the negative impact of low ruminal pH on the microbial population digesting fibre. With processed maize (dry rolled maize and high moisture maize) finishing diets, Mader *et al*. (1991) also found high correlation between diet NDF digestibility and gain ($r = 0.80$), intake ($r = 0.68$), and feed: gain ratios ($r = 0.66$) and further suggested that the ideal roughage source to complement finishing diets may be depended on the maize processing method used. Shain *et al*. (1999) observed significant elevation in ruminal pH, and higher A: P ratio with wheat straw diets compared to Lucerne hay diets with similar particle size in a metabolism trial. Shain *et al*. (1999) also observed significant higher ruminal DM % between all concentrate and corn cob diets in comparison to the hay diets. Results from a number of studies (Yang *et al*., 2001; Krause *et al*., 2002; Beauchemin *et al*., 2003; Kononoff *et al*., 2003; Plaizier, 2004) using the PSPS technology have not been conclusive enough to determine the effects of dietary peNDF on feed intake, chewing time, and ruminal pH. Beauchemin and Yang (2005) proved that maize silage with different peNDF content did not change ruminal pH in dairy cows, however the reduction in peNDF of maize silage in their study did show less chewing and rumination

time and suggest that peNDF content may be an important factor stimulating rumination. In support to the dairy research, results from Galyean and Defoor (2003) and Markham *et al*. (2004) suggested that particle size may not be a useful measurement of roughage value for feedlot diets and both researchers implicate NDF from roughage source as a useful index for substituting roughage in finishing diets. Galyean and Defoor (2003) data from trail-adjusted means suggest that dietary roughage level accounted for 69.9% of the variation in DMI while dietary NDF and eNDF supplied by roughage accounted for 92 and 93.1% respectively for variation in DMI. Thus, in conclusion, the CHDRM diet in this experiment, seem to have a high fragility value compared to the other diets and may not favour rumination and salivation to the same extent as the other diets which support Grant (2010) theory.

Table 5.3 The effect of roughage source on distribution of NDF fractions above 3.175mm for the four different experimental diets expressed on dry matter basis

	Experimental diets[1]			
Composition (%DM)	WSDRM	EHDRM	CHDRM	MSDRM
Total neutral detergent fibre >3.175mm	16.18	16.12	16.97	17.41
Total digestible neutral detergent fibre >3.175mm	12.44	12.68	13.74	14.66
Total neutral detergent fibre (roughage) >3.175mm	7.41	5.81	7.13	6.41

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)



[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

Figure 5.1 The effects of roughage source on distribution of NDF fractions above 3.175mm for the four different experimental diets

5.2 THE EFFECT OF ROUGHAGE SOURCE ON VOLATILE FATTY ACID COMPOSITION

5.2.1 Total ruminal volatile fatty acid concentration and composition

The molar concentrations of the three major VFA's produced by the experimental animals fed different sources of roughage in combination with dry rolled maize, are summarized in Table 5.4 and illustrated in Figure 5.2. In this experiment the total VFA concentrations ranged between 61.60 and 74.47 mM/L and were numerically lower compared to results found in experiment 1. This low total VFA and molar proportions compares to guidelines for VFA concentrations and molar proportions for cattle on 40% roughage and 60% concentrate diets (McDonald *et al*., 2002). The primary reason for lower production of VFA in this experiment is the diminishing effects of the constant low pH on growth and activity of rumen micro organisms, especially the cellulose fermenting bacteria. Ruminal starch digestion may also have been lower during this experiment since the extent of starch digestion for dry rolled maize in the rumen of steers is lower at 72% (Owens *et al.*,1986) compared to finely ground maize at 78% (Owens *et al*., 1986). Total VFA concentration for WSDRM diet was the lowest at 61.60 mM/L and differed from CHDRM and MSDRM diets ($P<0.05$). Reasons for this can be explained by a combination of both more rumination and

salivation with wheat straw which diluted the fermentation acids and lower the concentration of propionate. Sudweeks (1977) found that diets which increase chewing time and saliva flow have lower concentrations of VFA due to a dilution effect and increased acetate: propionate ratios which partly explained the significant lower concentration of total VFA and higher acetate: propionate ratio for the WSDRM diet ($P<0.05$) compared to the other experimental diets. However, another explanation would be a higher outflow rate of small grain particles (small pool size- lower sieve and bottom pan fractions on the PSPS) from the rumen which may have caused less extensive ruminal starch digestion with lower propionate and total VFA concentration. Molar ratio of acetate: propionate for WSDRM diet was 3.69 and differed from ratios of 2.37 and 2.55 for CHDRM and MSDRM diets respectively ($P<0.05$). The major difference seems to be the lower concentration of propionate in the WSDRM diet. This was also observed by Shain *et al*., (1999) for steers fed straw diets and these authors suggests altered starch digestibility and/or utilization of starch. Joanning *et al.* (1981) investigated grain and silage mixtures fed to steers and blame incomplete starch digestion as the major reason for the decreased VFA production efficiency. Depressions in NDF and starch digestibility expressed as a fraction of the total nutrients were 36 % and 53% respectively for immature maize silage vs. mature maize silage mixtures fed to steers (Joanning *et al*., 1981). Thus, stage of maturity of roughage which ultimately change digestibility of NDF from roughage again implicates the importance of roughage type and source on rumen fermentation characteristics. Essentially, the same observation was made in this experiment when comparing wheat straw, containing less dNDF with cottonseed hulls.

Table 5.4 Molar concentrations of volatile fatty acids produced over time in the rumen of steers fed different roughage sources in combination with dry rolled maize

	Experimental diets[1]				
	WSDRM	EHDRM	CHDRM	MSDRM	SEM[2]
Total VFA production (mM/l)	61.60[a]	72.29[ab]	82.75[b]	74.47[b]	3.33
Acetate (mM/l)	39.75[a]	45.05[ab]	47.45[b]	44.36[ab]	1.77
Propionate (mM/l)	10.97[a]	16.82[ab]	22.19[b]	17.79[ab]	2.53
Butyrate (mM/l)	9.61	8.88	11.33	10.85	0.96
A: P Ratio	3.69[a]	2.99[ab]	2.37[b]	2.55[b]	0.31
Acetate (%)	64.64[a]	62.74[ab]	57.83[b]	59.70[ab]	1.59
Propionate (%)	17.74	22.67	26.14	23.81	2.58
Butyrate (%)	15.52	12.43	13.87	14.49	1.14

[abc] Means in the same row with different superscripts differ ($P< 0.05$)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2] SEM = Standard error of the mean

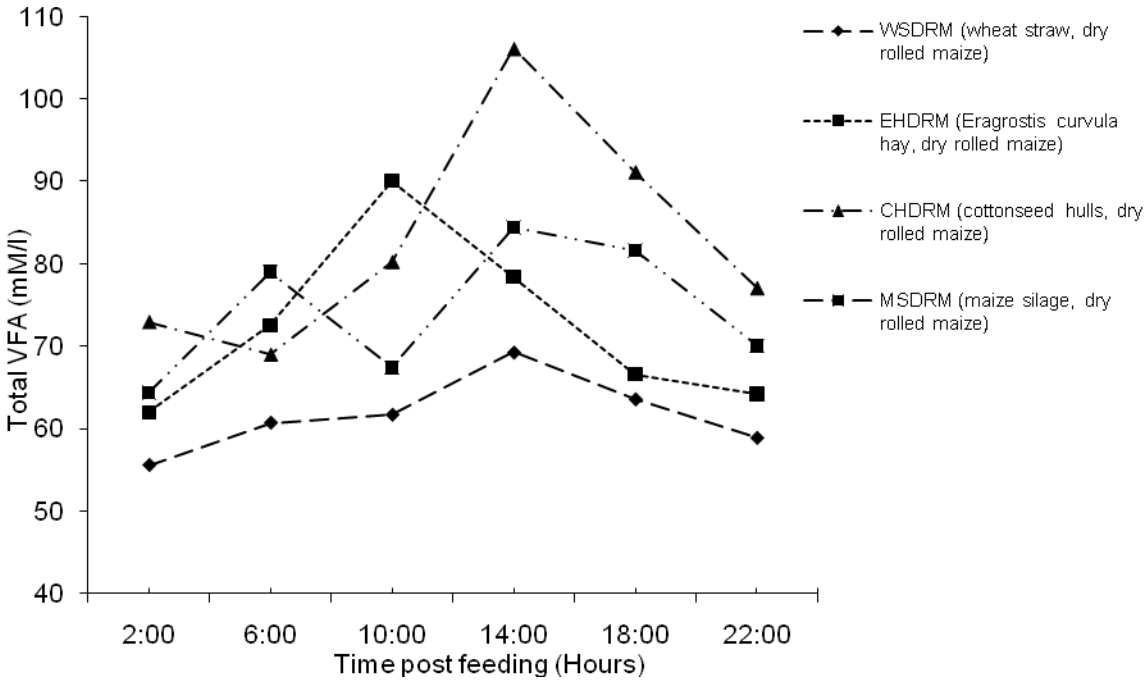


Figure 5.2 The effect of different roughage sources in combination with dry rolled maize on total volatile fatty acid concentrations produced in the rumen at different time intervals post feeding

5.2.2 Ruminal acetate concentration

Acetate concentration (Table 5.5 and Figure 5.3) for WSDRM was the lowest at 39.75mM/L and differed only from CHDRM with highest concentration of 47.45mM/L ($P<0.05$). The lower dietary fat content of the experimental diets in this study was lower at 2.5-3% compared to the fat content of diets with hominy chop based diets from the first experiment at 5-6% fat. This may have had less negative effects on fibre digesting bacteria since acetate as percentage of the total VFA concentration in this experiment was numerically higher than in experiment 1. Clary *et al*. (1993) reported A: P ratios ($P<0.05$) higher without supplemental fat compared to supplemental fat and total VFA concentrations similar to levels found from this study. Higher levels of acetate in this study can also be as result of a higher ruminal pH for animals consuming the WSDRM diet. Latham *et al*., (1974) suggested that the buffering action of saliva increases rumen pH, thereby favouring the synthesis of acetate over propionate. The CHDRM diet showed peaked production at 14 hours post feeding of 60.03mMol/L ($P<0.05$) which coincides with the time of peaked

total VFA concentration and lowest pH recording. This higher concentration of acetate can be attributed to the extent of digestion of the cottonseed hulls compared to the wheat straw, although in theory the buffering from saliva would have been less than with the WSDRM diet.

Table 5.5 Molar concentrations of acetate produced over time in the rumen of steers fed experimental diets containing different roughage sources in combination with dry rolled maize

	Experimental diets[1]				
	WSDRM	EHDRM	CHDRM	MSDRM	SEM[2]
Time	mM/l	mM/l	mM/l	mM/l	
2:00	35.55	37.72	42.90	39.55	2.38
6:00	39.37	44.74	41.06	49.03	3.91
10:00	39.68[a]	54.26[b]	46.95[c]	39.91[a]	1.59
14:00	44.33[a]	47.59[a]	60.03[b]	49.56[a]	2.20
18:00	41.14	43.07	50.58	46.72	2.87
22:00	38.44	42.91	43.20	41.38	2.81
Means	39.75[a]	45.05[ab]	47.45[b]	44.36[ab]	1.77

[abc] Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2] SEM = Standard error of the mean

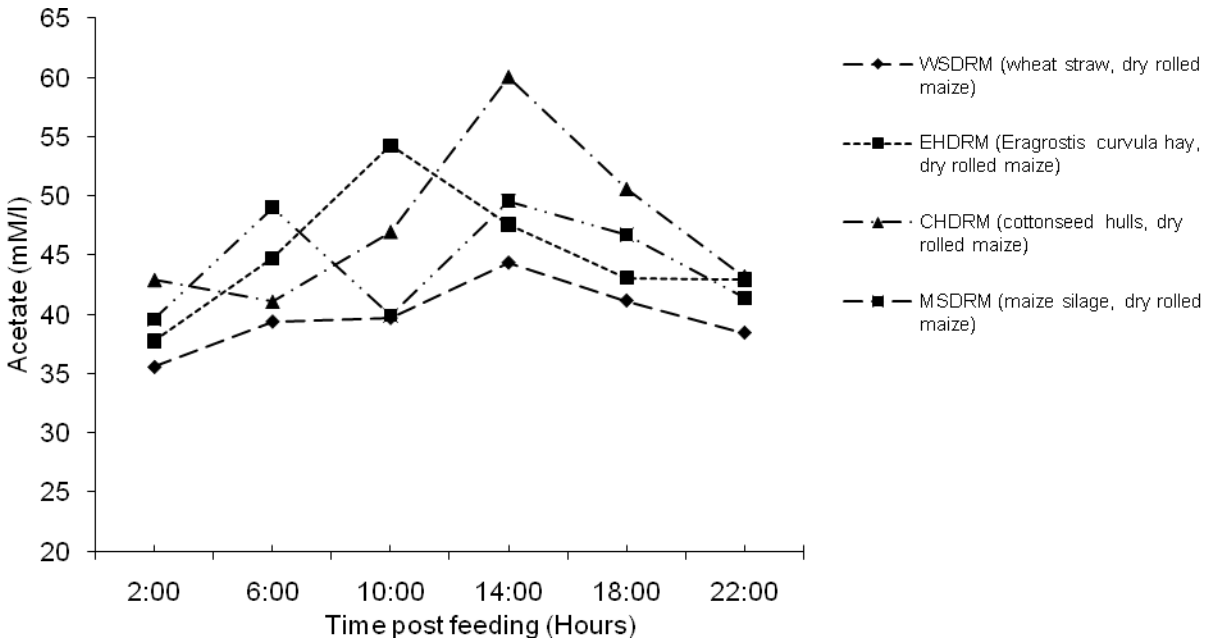


Figure 5.3 The effect of different roughage sources in combination with dry rolled maize on molar concentrations of acetate produced in the rumen at different time intervals post feeding

5.2.3 Ruminal propionate concentration

Ruminal propionate concentrations are listed in Table 5.6 and illustrated in Figure 5.4. Total propionate for WSDRM was the lowest at 10.97mM/L and differed only from the CHDRM diet ($P<0.05$). There was also an absence of a well defined peak concentration observed for propionate with the WSDRM diet in comparison to the other diets (Figure 5.4). This may be the result of the buffering action from salivation and consequently steady ruminal pH. Figure 5.4 also shows a very distinctive difference in the time intervals peak concentrations of propionate occurred for EHDRM, CHDRM and MSDRM diets. Reasons for this may be due to different rates of starch and /or fibre digestion as influenced by rumen pH and different rumination patterns as a result of the use of different roughage sources.

Table 5.6 Molar concentrations of propionate produced over time in the rumen of steers fed experimental diets containing different roughage sources in combination with dry rolled maize

	Experimental diets[1]				
	WSDRM	EHDRM	CHDRM	MSDRM	SEM[2]
Time	mM/l	mM/l	mM/l	mM/l	
2:00	10.92^{a}	14.17^{ab}	16.98^{b}	14.31^{ab}	1.59
6:00	11.07	17.22	16.24	17.72	2.20
10:00	10.81^{a}	24.24^{b}	20.32^{abc}	15.96^{a}	2.26
14:00	11.72^{a}	20.36^{ab}	29.81^{b}	20.04^{ab}	3.05
18:00	11.10^{a}	13.72^{ab}	27.88^{b}	21.17^{ab}	4.37
22:00	10.18^{a}	11.20^{ab}	21.93^{b}	17.53^{ab}	3.46
Means	10.97^{a}	16.82^{ab}	22.19^{b}	17.79^{ab}	2.53

^{abcd} Means in the same row with different superscripts differ ($P< 0.05$)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2]SEM = Standard error of the mean

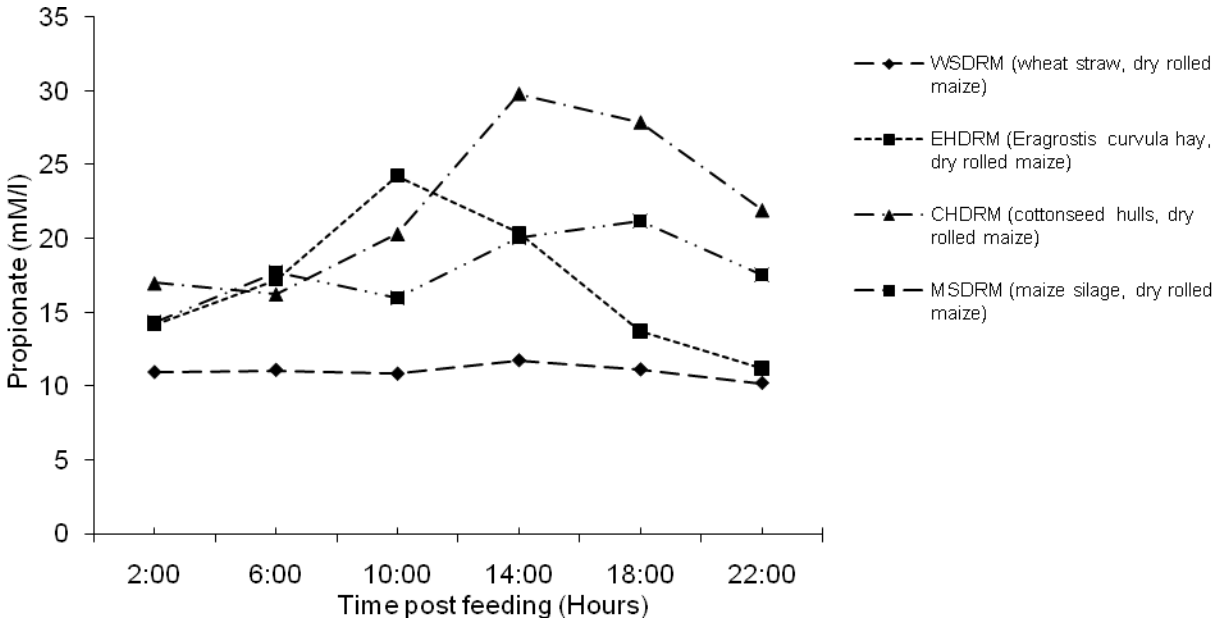


Figure 5.4 The effect of different roughage sources in combination with dry rolled maize on the molar concentrations of propionate produced in the rumen at different time intervals post feeding

5.2.4 Ruminal butyrate concentration

No differences were observed for butyrate concentration (Table 5.7 and Figure 5.5) for all four experimental diets (P>0.05). However, concentrations were 52 and 75 percent lower than recorded in experiment 1 for diets containing *Eragrostis curvula* hay and wheat straw respectively. Butyrate as regulator for intake *per se* can be eliminated since butyrate is normally metabolized in the rumen epithelium to B-hydroxybutyrate (McDonald *et al*., 2002). Interestingly, butyrate is the only VFA to be used as an energy source by rumen papillae and mitotic indices of the rumen papillae in sheep increased by between 1.29 and 2.65% after administration of sodium n-butyrate intraruminally (Sakata and Tamate, 1978). However butyrate enhances papillae proliferation *in vivo* but not *in vitro* (Baldwin, 1999). The reason for this may be imbedded in the fact that insulin growth like factors (IGF-1) in the rumen are responsible for these changes in proliferation through a cascade of physiological changes in the epithelium. Dietary energy–dependent alterations of rumen morphology and function are accompanied by corresponding changes in systemic IGF-1 and ruminal IGF-1R (Shen *et al*., 2004).

Table 5.7 Molar concentrations of butyrate produced over time in the rumen of steers fed experimental diets containing different roughage sources in combination with dry rolled maize

	Experimental diets[1]				
	WSDRM	EHDRM	CHDRM	MSDRM	SEM[2]
Time	mM/l	mM/l	mM/l	mM/l	
2:00	7.72[a]	8.54[a]	11.34[b]	9.14[ab]	0.82
6:00	8.94	9.05	10.29	10.86	1.15
10:00	9.93	9.84	11.21	10.18	1.05
14:00	11.93[abc]	8.78[a]	14.13[bc]	13.09[c]	1.24
18:00	10.02	8.36	10.91	12.13	1.43
22:00	9.11	8.73	10.13	9.69	1.33
Means	9.61	8.88	11.33	10.85	0.96

[abc] Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2] SEM = standard error of the mean

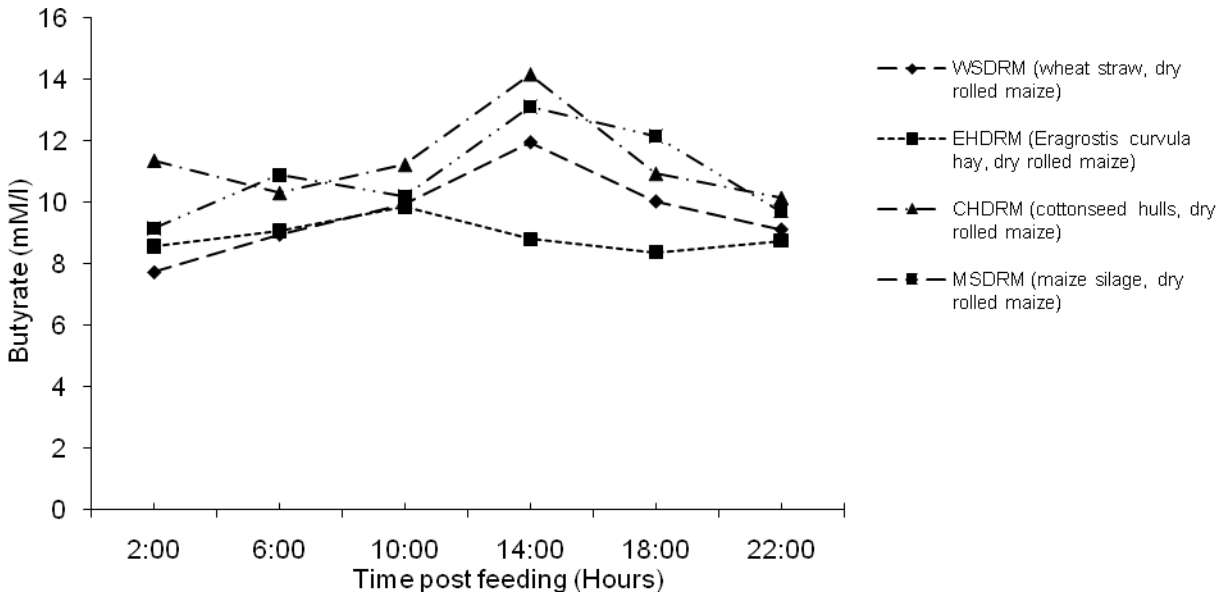


Figure 5.5 The effect of different roughage sources in combination with dry rolled maize on molar concentrations of butyrate produced in the rumen at different time intervals post feeding

5.2.5 General discussion on volatile fatty acid concentrations

Observed total volatile fatty acid concentrations in this experiment is approximately 30% lower compared to the total ruminal VFA concentrations in experiment 1. Peak VFA concentration was 14 hours post feeding for all diets except EHDRM that peaked at 10 hours. These observations can be explained by lower ruminal digestion of starch for dry rolled maize compared to hominy chop in the first experiment. In the review article by Owens (2006), the author categorise different processing methods for maize. The total-tract starch digestibility is higher for rolled and ground maize than whole maize (Figure 5.6). As can be seen in Figure 5.6 (Owens, 2006), substantial amounts of starch were digested in the small intestine and large intestine of steers for dry rolled maize proportional to high moisture maize with high ruminal digestion. Adequate data on ruminal digestion values for South African hominy chop is very limited but one can assume very high values possibly due to the smaller particle size of the starch. Grinding maize to a smaller particle size increases the extent of starch digestion, both in the rumen and total digestive tract. Average across 4 trials with lactating dairy cows fed dry rolled grain, starch digestibility increased by 2.7 (+8)% (P=0.023) for each decrease in mean particle diameter of 1000 microns (Owens, 2006).

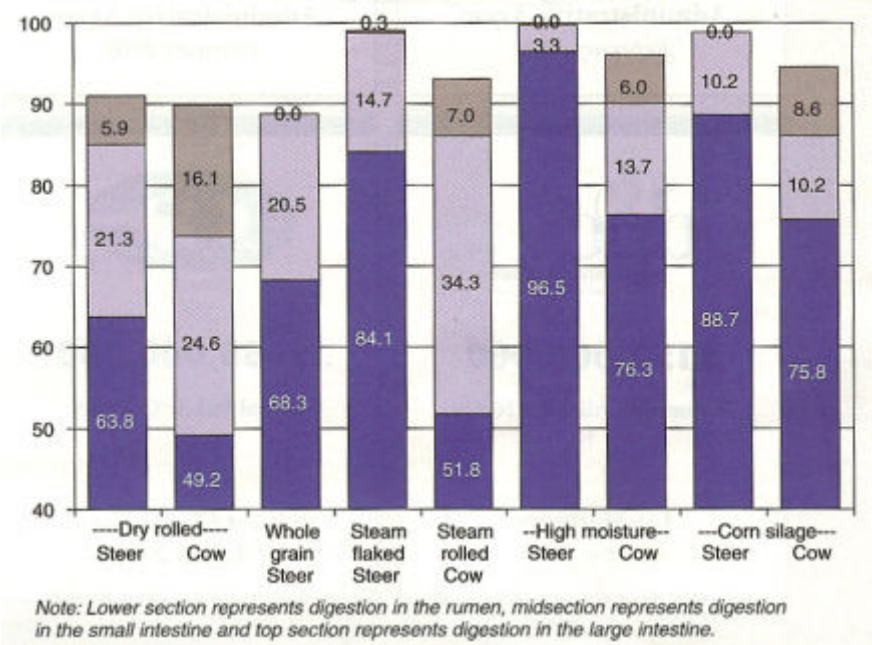


Figure 5.6 Site of starch digestion (% of dietary starch) from maize grain and maize silage fed to steers and cows (Owens, 2006)

5.3 THE EFFECT OF ROUGHAGE SOURCE ON RUMEN AMMONIA NITROGEN CONCENTRATIONS

The effects of roughage sources in combination with dry rolled maize on rumen NH_3-N concentration at various time intervals are presented in Table 5.8 and Figure 5.7. Gradual decrease in NH_3-N concentration can be seen in all treatment diets from levels between 12.09 and 13.93mg NH_3-N/100ml to levels between 4.72 and 6.87mg NH_3-N/100ml. Ammonia nitrogen concentrations 6 to 12 hours post feeding were above the critical threshold of 5 mg/100ml needed for maximum microbial protein synthesis (Satter and Slyter, 1975). No differences were observed between experimental diets for total NH_3-N concentration over the 24 hour observation period ($P>0.05$). The concentrations of NH_3-N observed during this experiment were higher than was observed in experiment 1. Reasons for this may be explained by the need for microbial populations to utilize ammonia. Fibre digesting bacteria mainly require ammonia whilst non fibre fermenting bacteria which primarily ferment starch, sugar and soluble fibre can utilize ammonia, amino acids and peptides as a nitrogen source (Russel *et al*., 1992). Hominy chop diets in the first experiment contained more NDF and consequently more dNDF than the dry rolled maize diets. Thus, the need for the microbial populations to utilize more NH_3-N during the first experiment partly explains the lower concentrations observed. Absence of NH_3-N concentration differences in the current experiment is probably due to similar concentrations of dietary crude protein and amino acid concentration with similar nitrogen solubility. Similar results were reported by Marshall *et al*., (1992) where different sources of roughage were substituted in diets containing equal crude protein and starch levels.

Table 5.8 The effect on NH_3-N concentrations produced over time in the rumen of steers fed the experimental diets containing different roughage sources in combination with dry rolled maize

	Experimental diets[1]				
	WSDRM	EHDRM	CHDRM	MSDRM	SEM[2]
Time	mg NH_3-N/100ml	mg NH_3-N/100ml	mg NH_3-N/100ml	mg NH_3-N/100ml	
2:00	12.09	11.35	13.24	13.93	1.50
6:00	10.03	7.91	9.54	8.91	2.07
10:00	9.46	7.16	7.00	5.66	1.61
14:00	7.83[ab]	5.40[a]	10.22[b]	5.53[ab]	1.42
18:00	7.55	7.19	6.96	5.60	1.41
22:00	6.87	6.23	7.95	4.72	1.28
Means	8.97	7.54	9.15	7.39	1.09

[abc] Means in the same row with different superscripts differ ($P< 0.05$)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2] SEM = standard error of the mean

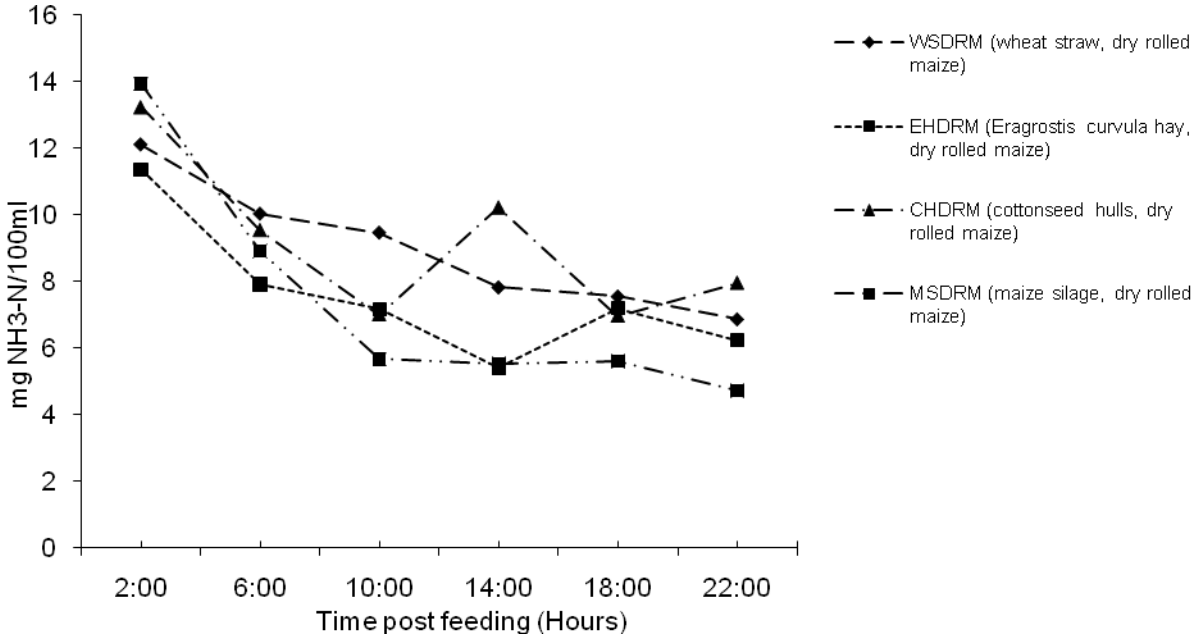


Figure 5.7 Effect of different roughage sources in combination with dry rolled maize on NH_3-N concentrations produced in the rumen at different time intervals post feeding

5.4 THE EFFECT OF ROUGHAGE SOURCE ON LACTIC ACID CONCENTRATION

The effects of roughage source on lactic acid concentration at various time intervals are presented in Table 5.9 and Figure 5.8. Peak lactic acid production for all experimental diets were between 14 and 22 hours post feeding with EHDRM being the highest at 18 hours post feeding with 4.66 mg/100ml (0.52mM/l) and differed from all other treatment diets ($P<0.05$). Although EHDRM diet had higher lactic acid concentrations, absence of a depression in VFA concentrations compared to the other diets clearly indicated that lactic acid concentrations were not high enough to decrease ruminal pH and alter the rumen microbial population. The mean total lactic acid concentration observed during the 24 hour time period was the highest for EHDRM diet at 3.79 mg/100ml and differed only from WSDRM and MSDRM diets with 2.77 and 2.85 mg/100ml respectively ($P<0.05$). These lactic acid concentrations were almost 15 to 87% higher than concentrations observed for experiment 1. Lactic acid concentration (Table 5.9 and Figure 5.8) in this experiment may have contributed substantially more towards low ruminal pH values compared to values reported in experiment 1, since peak lactic acid concentrations and time of lowest ruminal pH values coincide (Table 5.10 and Figure 5.9). Lactate is a 10 times stronger acid than VFA's and was traditionally

viewed as the culprit in acute forms of acidosis. However, lactate does not accumulate under normal conditions in the rumen and concentrations are usually below 5 µM but concentrations exceeding 40 mM are indication of severe acidosis (Owens *et al*., 1998). In this study, mean concentrations of lactic acid equates to concentrations between 0.31 - 0.42mg/l. This in turn would equate to levels of between 3 – 4 mM in the rumen which would place all these animals consuming the experimental diets in this experiment in a mild form of acidosis. Volatile fatty acids does not usually accumulate at high enough concentrations to reduce rumen pH drastically but when rate of production exceeds rate of absorption, depression of ruminal pH below 5 can be observed even without the accumulation of lactic acid (Britton & Stock, 1987). When ruminal pH decrease to pH 5.0 during acidosis, ionization of acids increases slightly, but added lactate is primarily responsible for increased hydrogen ion concentration. Lactate can depress pH more than similar amounts of other VFA because its pK value is lower than VFA's (3.8 vs. 4.8) and low pH or acidity drives the conversion of pyruvate to lactate through enhanced lactate dehydrogenation activity (Owens *et al*., 1998). In the case of CHDRM and MSDRM diets, both these experimental diets resulted in ruminal pH values near 5.0 for short periods of time (Table 5.10 and Figure 5.9). In comparison with hominy chop based diets of experiment 1, VFA concentrations in this study was numerically lower but lactic acid concentrations were higher than concentrations observed in experiment 1. Lactic acid is a 10 fold stronger acid than VFA and may have elevated the total acid load in the rumen more than during experiment 1 with hominy chop.

Table 5.9 The effect of different roughage sources on concentrations of lactic acid produced in the rumen at different time intervals

	Experimental diets[1]				
	WSDRM	EHDRM	CHDRM	MSDRM	SEM[2]
Time	mg/100ml	mg/100ml	mg/100ml	mg/100ml	
2:00	2.45^{a}	2.68^{a}	3.63^{b}	2.68^{a}	0.27
6:00	2.62	3.60	2.84	2.38	0.40
10:00	2.60^{a}	3.93^{b}	2.49^{a}	2.35^{a}	0.26
14:00	3.00^{a}	4.18^{b}	2.92^{a}	2.77^{a}	0.24
18:00	3.28^{a}	4.66^{b}	3.20^{a}	3.30^{a}	0.19
22:00	2.70	3.72	3.65	3.64	0.53
Means	2.77^{ac}	3.79^{b}	3.12^{abc}	2.85^{c}	0.26

^{abc} Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

2 SEM = standard error of the mean

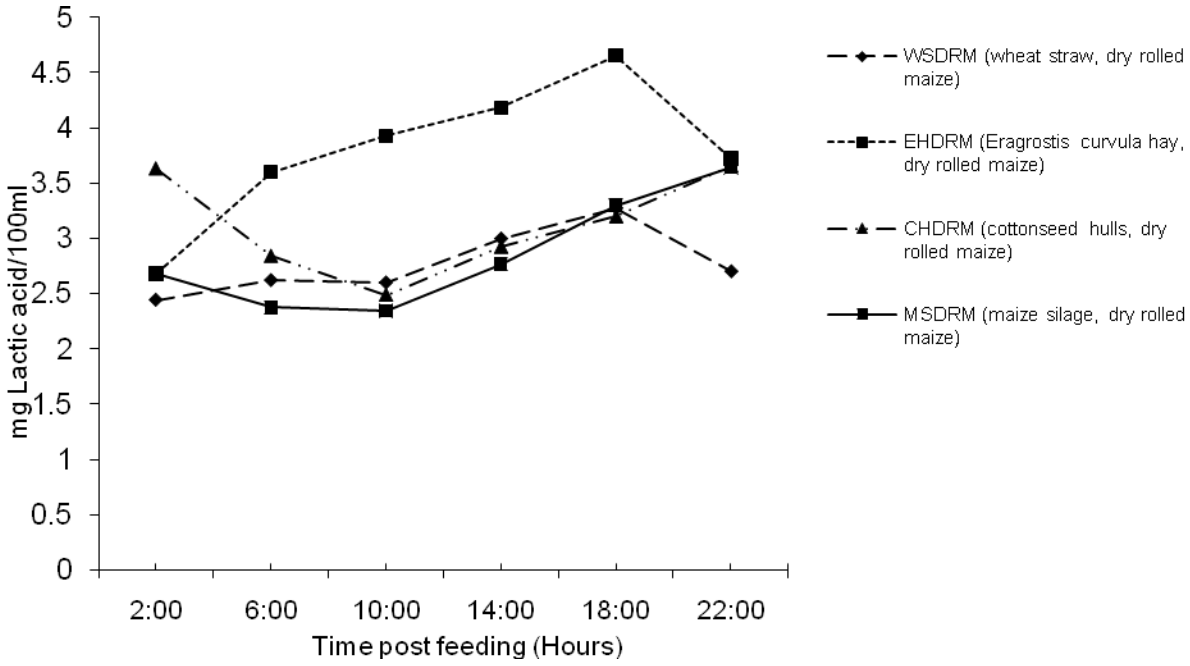


Figure 5.8 The effect of different roughage sources in combination with dry rolled maize on lactic acid concentrations produced in the rumen at different time intervals post feeding

5.5 THE EFFECT OF EXPERIMENTAL DIETS ON RUMEN pH

The values for ruminal pH in steers fed the experimental diets are presented in Table 5.10 and illustrated in Figure 5.9. Values for all treatments gradually decreased within the first 14 to 18 hours post feeding opposed to 10 hours post feeding for experiment 1. Mean pH value for WSDRM diet was highest at pH 6.10 ($P<0.05$) and differ only from the CHDRM and MSDRM diets with pH values of 5.65 and 5.53 respectively. This was probably due to more rumination and salivation elicited by diet characteristics, which in turn buffered the rumen in these experimental animals. MSDRM diet had the lowest mean pH value of 5.53 but only differed from the WSDRM ($P<0.05$). Lowest pH values for the 24 hour period coincide with the time when total VFA concentrations peaked (Figure 5.2) for all experimental diets. However, ruminal pH is related to VFA concentration (Erdman, 1988; Stokes *et al*., 1991) and the reduced

rate of VFA absorption resulted in a reduction in ruminal pH (Owens *et al.,* 1998). Ruminal pH below 5.6 is generally regarded as sub acute acidosis and ruminal pH below 5.2 regarded as acute acidosis (Cooper & Klopfenstein, 1996). The length of the time period spend below these pH thresholds values during a 24 hour period may be of more importance to explain the severity and proliferations of sub-acute or acute acidosis in animals than low pH value alone. However, the length of time is not well defined by researchers and needs further investigation.

Table 5.10 Effect of different roughage sources in combination with dry rolled maize as energy source on the ruminal pH at two hour intervals post feeding

	Experimental diets[1]				
Time	WSDRM	EHDRM	CHDRM	MSDRM	SEM[2]
0:00	6.35	6.32	6.25	6.28	0.13
2:00	6.25	6.05	6.28	5.90	0.14
4:00	6.20	6.05	6.13	5.73	0.16
6:00	6.23^{a}	5.88^{ab}	5.75^{b}	5.45^{b}	0.14
8:00	6.28^{a}	5.75^{ab}	5.70^{ab}	5.58^{b}	0.17
10:00	6.00	5.75	5.45	5.38	0.20
12:00	5.98^{a}	5.80^{ac}	5.28^{bc}	5.40^{c}	0.13
14:00	6.08^{a}	5.58^{abc}	5.15^{b}	5.30^{c}	0.17
16:00	5.98^{a}	6.00^{a}	5.30^{bc}	5.30^{c}	0.17
18:00	5.83	5.83	5.55	5.05	0.26
20:00	6.07	5.93	5.30	5.35	0.24
22:00	6.22	6.00	5.63	5.68	0.19
Means	6.10^{a}	5.90^{abc}	5.65^{bc}	5.53^{c}	0.13

^{abc} Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2]SEM = Standard error of the mean

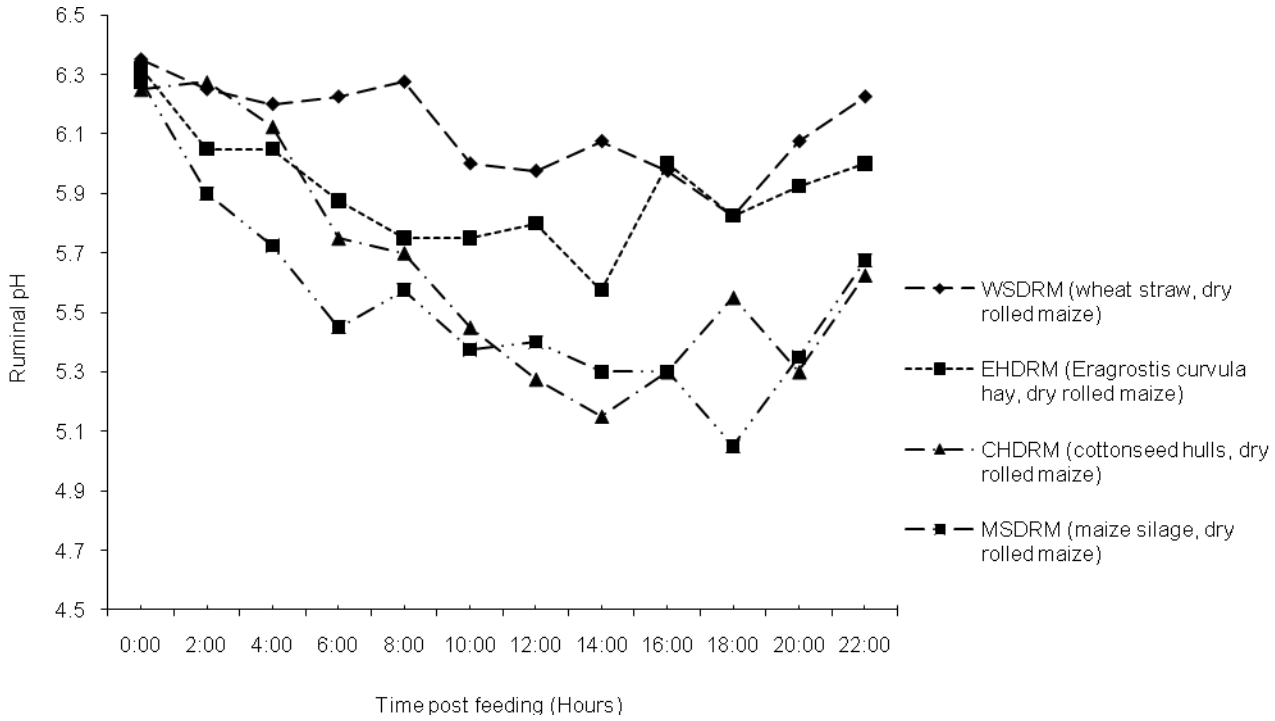


Figure 5.9 The effect of different roughage sources in combination with dry rolled maize on variation of pH in the rumen at different time intervals post feeding

Table 5.11 and Figure 5.10 summarise the effect of all treatment effect on duration of time spend below ruminal pH 5.6 and pH 5.2. Total duration in minutes spend below pH 5.6 for WSDRM were numerically lower at 103.75 minutes compared to the other treatment diets, but WSDRM treatment diet only differed from the MSDRM diet ($P<0.05$). Duration in minutes spend below pH 5.2 for WSDRM (10 minutes) also only differed from MSDRM (306.25 minutes) ($P<0.05$) although this time differ numerically from both EHDRM and CHDRM. This observation together with the 24 hour ruminal pH observation, DMI and VFA production indicates more rumen buffering from roughage source itself due to possible increased salivation and rumination from wheat straw and *Eragrostis curvula* hay. Campbell *et al*. (1992) reported lower chewing activity due to eating and rumination by steers fed corn cob with concentrate than traditional hay with concentrate diets. Decreased chewing activities would be associated with decreased salivary and secretion of buffers for neutralization of ruminal pH and VFA concentration. Explanation for the extended duration of time spend below pH 5.6 and 5.2 for CHDRM and MSDRM, is due to higher acid load from total VFA production (82.75mM/L and 74.47mM/L for CHDRM and MSDRM respectively vs. 61.6 mM/L for WSDRM) as well as lactic acid load peaks, as can be seen from Figure 5.7.

Table 5.11 The effect of different roughage sources on the time periods spent below pH thresholds 5.6 and 5.2 respectively during a 24 hour period post feeding

	Experimental diets[1]				
	WSDRM	EHDRM	CHDRM	MSDRM	SEM[2]
Time	Minutes	Minutes	Minutes	Minutes	
Minutes < pH 5.6	103.75[a]	248.75[ab]	610[ab]	703.75[b]	163.93
Minutes <pH 5.2	10[a]	42.5[ab]	160[ab]	306.25[b]	84.70

[abc] Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

[2]SEM = Standard error of the mean

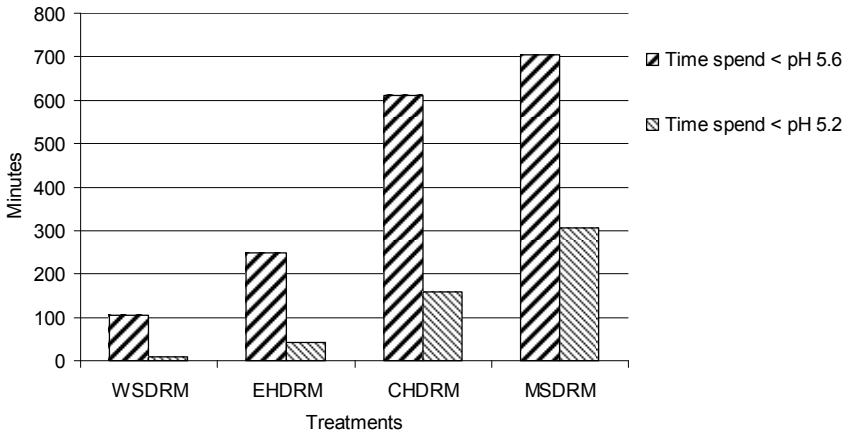


Figure 5.10 The effect of different roughage sources in combination with dry rolled maize on the time period spent below threshold pH 5.6 and 5.2 respectively during the 24 hour period

Ruminal pH readings revealed two distinctive patterns for all four diets. The diets WSDRM and EHDRM had very similar patterns (Fig. 5.9) compared to CHDRM and MSDRM which had constant lower values than the first mentioned diets. In the study by Markham *et al*. (2004), steers fed finishing diets with cotton seed hulls as roughage experienced more decrease in ruminal pH over time than steers fed Lucerne hay diets. This was also evident in the current experiment with CHDRM diet when considering the time spent below pH 5.6 and pH5.2 as well as mean ruminal pH.

5.6 THE EFFECT OF EXPERIMENTAL DIETS ON DRY MATTER INTAKE.

The DMI of the respective experimental diets is listed in Table 5.12. The DMI for WSDRM diet was lowest and differed from CHDRM diet (P<0.05). Intakes between WSDRM, EHDRM and MSDRM did not differ in this experiment. Dry matter intake for animals fed the MSDRM diet was not as severely depressed as DMI of animals consuming MSHC in experiment 1. Voluntary feed intake during this experiment was numerically lower than observations for DMI during the first experiment. This was due mainly because the experiment was conducted in the hotter part of the season and experimental animals were reaching mature body weight and had increased carcass fat as the result of the prolonged period on finishing diets from the previous experiments. There is sufficient evidence to substantiate this claim since body fatness and heat exposure reduced feed intake (McDonald *et al*., 1988).

Table 5.12 Effect of different roughage sources and dry rolled maize as energy source on dry matter intake (DMI) of experimental animals

	Experimental diets[1]				
DMI (kg/kg $LW^{0,75}$ /day)	WSDRM	EHDRM	CHDRM	MSDRM	SEM[2]
Means	0.082[a]	0.090[ab]	0.098[b]	0.091[ab]	0.004

[abc] Means in the same row with different superscripts differ (P< 0.05)

[1] Experimental diets: WSDRM (wheat straw, dry rolled maize); EHDRM (*Eragrostis curvula* hay, dry rolled maize); CHDRM (cottonseed hulls, dry rolled maize); MSDRM (maize silage, dry rolled maize)

2SEM = Standard error of the mean

5.6 THE INFLUENCE OF ROUGHAGE SOURCE ON THE INCIDENCE OF BLOAT

The effect of roughage source on incidence of frothy bloat was evident in only one experimental diet. Cottonseed hulls in combination with dry rolled maize showed clinical signs of frothy bloat in every experimental animal for all the periods during this experiment. Ruminal contents are typically stratified and partially digested feed particles are readily discernable in the digesta of animals in which gas has separated normally from fluid and particles (Cheng *et al*., 1998). This can clearly be seen in Figure 5.10 for the MSDRM diet. In contrast, ruminal content in animals suffering from frothy bloat form a dispersion of gas, liquid and feed particles (no "filter bed" effect) and can be seen in Figure 5.11 and Figure 5.12. With feedlot bloat, the foam-producing agents seem to be mainly of microbial origin (Cheng *et al.*, 1976). High levels of soluble protein contribute to stable foam. Not all animals with frothy rumen contents will bloat and this is evident in this case, as rumen cannula would have dislocated from the rumen orifice if the pressure exceeded normal pressure, which happens often in some rumen cannulated animals with bloat. Low ruminal pH and conditions of acidosis are usually associated with bloat but bloat can occur when pH of ruminal fluid is above 6.0 (Sakauchi and Hoshino, 1981a as sited by Cheng *et al*., 1998). The release of

intra-cellularly stored carbohydrates upon cellular breakdown contributes to an increased viscosity of ruminal fluid in addition to the extracellular slime.

Figure 5.11 Maize silage used as roughage source in combination with dry rolled maize. Note the very distinctive stratification in the rumen

Figure 5.12 Cottonseed hulls used as roughage source in combination with dry rolled maize. Note the severity of frothy bloat in this animal and the total absence of distinctive rumen content stratification

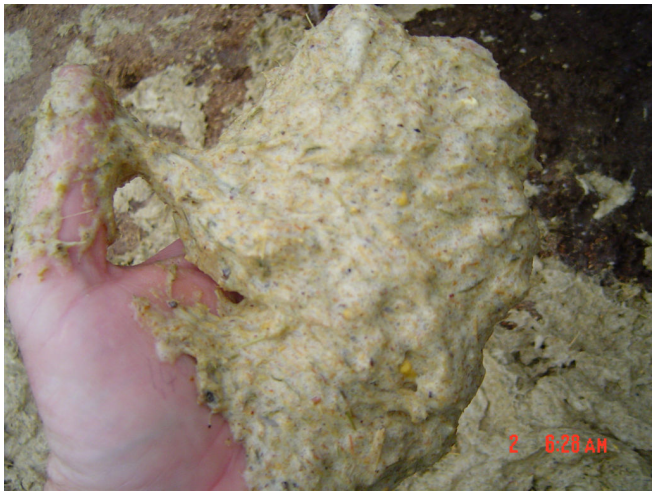


Figure 5.13 Close observation of rumen content shown very little intact fibre from cottonseed hulls with very foamy consistency

CHAPTER 6.

CONCLUSION

This study was conducted to evaluate the typical feedlot finishing diets fed in South African feedlots. The objective of this study was to investigate the effects of four different roughage sources in combination with hominy chop or dry rolled maize. Although the experimental diets were formulated on an iso CP; NDF; and starch in both experiments, ruminal fermentation characteristics were different for different roughage source: energy source combinations. In all instances these formulations could be considered as practical under feedlot conditions. The substitution of roughage and energy sources as was done in these experiments is a common practise in South African feedlots. The by-products like hominy chop and roughage sources like *Eragrostis curvula* hay and wheat straw are almost always subjected to seasonal production and availability. Hominy chop may also be replaced by maize depending on price and availability from the milling industry. Despite the ruminal fermentative differences between the hominy chop diets and dry rolled maize diets as a result of different forms of NSC and level, there was also observed ruminal fermentative differences between diets containing different roughage sources. Differences in particle size of the grain or by-product (hominy chop) fractions had a major influence on ruminal fermentation and VFA concentrations. Since the majority of ruminally produced VFA are produced from fermentable starch in high concentrate diets, the smaller the particle size of the grains, the more exposure of starch surface area is available for microbial attack and enzymatic action. Roughage source *per se*, did have an effect on rumen fermentation characteristics and ruminal pH during these experiments. Although no chewing and (or) rumination measurements have been collected during these experiments, roughage particle size, especially the large pool size did show some relationship between fragility of the roughages based on dNDF values expressed as a percentage of total NDF for these pool sizes and rumen pH. The results from these experiments also indicate that particle size of the diets *per se* did not influence rumen fermentation to the same extent as NDF source. The roughage sources (therefore NDF sources) particularly influence ruminal pH and length of time periods spend below pH 5.6 and 5.2 respectively through buffering action during rumination and salivation. In both these experiments, VFA production did have an impact ruminal pH, with lactate production contribution towards low ruminal pH especially in experiment 2 being more evident than in experiment 1. The 30% higher concentrations of VFA produced with hominy chop diets in experiment 1 and concurrently lower rumen ammonia nitrogen concentrations compared to that of the dry rolled maize diets indicates a higher ruminal microbial activity. This may be explained by higher NDF and digestible NDF levels in the hominy chop diet compared to the dry rolled maize diet as well as the higher metabolisable energy level and higher digestibility of starch entering the rumen from the hominy chop diet.

Generally, the rumen NH_3-N concentrations in the experiment where hominy chop was used as energy a source were rapidly depleted compared to dry rolled maize diets 6 to 12 hours post feeding. This indicated a higher demand for NH_3-N by the microbial populations in the rumen when feeding rations containing hominy chop as energy source. Thus more rumen degradable protein may be needed in these rations to optimize efficiency of the microbial population.

IMPLICATIONS

Results from these experiments indicate that roughage sources like wheat straw and *Eragrostis curvula* hay included on equal NDF basis in formulations, stimulate rumination and salivation more compared to cottonseed hulls and maize silage in combination with hominy chop and (or) dry rolled maize. Using formulations with cotton seed hulls included on an equal NDF basis in combinations with hominy chop or with dry rolled maize, have the tendency to produce high levels of VFA without adequate stimulation of rumination and salivation to the same extent as wheat straw and *Eragrostis curvula* hay to buffer the rumen and keep ruminal pH optimal for microbial activity. This may result in a higher acid load in the rumen and may cause metabolic disorders like bloat, sub-acute ruminal acidosis or acute ruminal acidosis. Maize silage are different in comparison to the other roughage sources due to the fact that silage contains energy in the form of grains and VFA's with approximately half of maize silage contributing to roughage on a dry basis. Thus, replacing roughage on an equal NDF basis, maize silage will contribute a portion of highly rumen degradable starch and VFA's to the diet and this always replace some of the energy from hominy chop or maize. This substitution of grain from roughage limits the inclusion of maize or hominy chop in the diet and ultimately limits the starch load into the rumen. Extent of ruminal starch digestion will increase with processing of the grain. When grain processing methods like dry rolling or grinding are inconsistent, shifts in rumen fermentation characteristics can result in acidosis due to rapid and excessive increase of produced fermentation acids. The finer the particle size of the starch components, the more likely it is to increase the acid load in the rumen. This places more emphasis on the roughage source ability to stimulate rumination and salivation to buffer the produced acids through the secretion of bicarbonate. Substitution of hominy chop with dry rolled maize will shift some digestion of starch from the rumen to the small intestines. This may be advantages because shifting the metabolic pathway away from fermentation of energy to VFA production in the rumen rather to glucose absorption via the intestines, increase the efficiency of utilization of ME and ultimately NEg.

Caution should be taken when replacing hominy chop with maize, especially if the particle size of the processed maize is small. The results found in these experiments confirm the theory and observations from other researchers that certain roughage sources may be better in combination to a specific energy source than others. From our results obtained from these experiments, roughage sources like wheat straw

and *Eragrostis curvula* hay may stimulate rumination and salivation more and buffer the rumen better when feeding high concentrate finishing diets compared to roughages like cottonseed hulls and maize silage. This may warrant the inclusion of roughages with low fragility (high NDF levels with low digestible NDF values) like wheat straw and *Eragrostis curvula* hay in feedlot finishing diets when timely feed delivery practises in a feedlot are not well managed. There are practical considerations with the inclusion of *Eragrostis curvula* hay as a roughage source in total mixed rations. In practise this hay doesn't blend in well with other feed ingredients unless it is cut to a very fine homogeneous particle size. Under almost all circumstances, fine particles of especially the energy source will separate from the ration regardless of mixing time or mixer. The implication of this can be detrimental to normal eating behaviour and rumen function since it unintentionally forces animals into selectively consuming hay and concentrate.

CHAPTER 7.

CRITICAL EVALUATION

In order to set standards for the Penn State Forage Particle Separator to be used as an accurate measurement instrument for feedlot diets, a wide range of diets needs to be tested for each roughage source in combination to an energy source. Rumen fermentation characteristics need to be assessed on each diet with differing roughage processing methods in combination with different grain processing methods or by-products. Changes to the experimental design in order to conduct both experiments at the same time would have eliminated the consequences of animal physical maturity, level of body fat and its negative impacts on DMI. Higher DMI reflect in a higher VFA concentration. This would have required 8 experimental animals to do this same study. Having done these two experiments according to design protocol, comparisons between experiment data sets was difficult to be made because of the above mentioned problem as well as change in season and duration of this study. In order to counteract the difference in daylight hours and the effect on DMI, I would recommend commencing trial work in October and be finished by March the next year. The observations with regards to NDF in this study could have been better explained if *in vivo* digestibility studies were conducted on the roughage source in question by the *in situ* technique. The use of markers for solid and liquid passage rates could have explained much of the difference in rumen fermentation characteristics and especially the ruminal retention time of roughage particles. Ruminal pH differences could have also been better explained between roughage source and energy source if ruminal osmolality was measured during these experiments. Rumen osmolality explains dissociation constants of VFA and also free hydrogen concentration in the rumen which ultimately relates to pH. No special focus on ruminal microbial protein production was made in this study and in future studies methods to observe microbial protein production like purine derivatives (allantoin and uric acid) may be of great value for the researcher to determine microbial population activity and fermentation trends. Chewing and rumination parameters (number of chews/ hour and Saliva flow) were not accessed during this trial and could be valuable information to explain fermentation patterns and the effect of roughage source on ruminal pH.

Planning concurrent commercial studies in feedlot environments to determine animal performances on these different roughage sources in combination with energy source can be of great value and performance data can be compared to metabolism data. Net energy gains; dry matter feed conversion ratios; digestive upsets; incidence of liver abscess; and damage to rumen wall epithelium could also be valuable information in these studies to further support the findings of the current study.

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