The Effect of Sugar, Starch and Pectin as Microbial Energy Sources on *In Vitro* Forage Fermentation Kinetics

by

Marcia Malan

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Animal Science)

at

Stellenbosch University

Department of Animal Sciences

Faculty of AgriScience

Supervisor: Prof CW Cruywagen

Date: *March 2009*

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: ……

Abstract

Title: The Effect of Sugar, Starch and Pectin as Microbial Energy Sources on *In Vitro* Forage Fermentation Kinetics

Name: Marcia Malan

Supervisor: Prof. C.W. Cruywagen

Institution: Department of Animal Sciences, Stellenbosch University

Degree: MScAgric

Ruminants have a compound stomach system that enables them to utilize forages more efficiently than monogastric animals. However, forages alone do not contain sufficient nutrients to meet the requirements of high producing dairy cows. Forages are high in fibre and their nutrient availability depends on the degree of cell wall degradability. Improvements in forage fermentation would increase energy intake and subsequently milk production and performance by dairy cows. It is therefore important to find ways to improve forage degradation and utilization in the rumen.

The use of different non-fibre carbohydrate (NFC) sources has different effects on animal performance. Supplementing forage based diets with energy sources containing sugar, starch or pectin results in variation in performance measurements such as milk yield, milk composition and dry matter intake (DMI).

This thesis reports on two studies in which the effect of energy supplementation on forage fermentation and digestion parameters was investigated. In the first study an *in vitro* gas production protocol was used to determine the effect of sugar (molasses), starch (maize meal) and pectin (citrus pulp) on total gas production and rate of gas production of different forages. The forage substrates included wheat straw (WS), oat hay, (OH) lucerne hay (LUC), ryegrass (RYE) and kikuyu grass (KIK). The three energy sources, as well as a control (no energy source) were incubated *in vitro* with each of the above mentioned forages. Rumen fluid was collected from two lactating Holstein cows receiving a diet consisting of oat hay, lucerne, wheat straw and a concentrate mix. Forages alone (0.25 g DM) and/or together (0.125 g DM) with either molasses (0.1412 g DM), citrus pulp (0.1425 g DM) or maize meal (0.125 g DM) were weighed into glass vials and incubated for 72 hours. The weights of the energy sources were calculated on an energy equivalent basis. Blank vials, that contained no substrates, were included to correct for gas production from rumen fluid alone.

The substrates were incubated in 40 ml buffered medium, 2 ml of reducing solution and 10 ml rumen fluid. Gas pressure was recorded automatically every five minutes using a pressure transducer system and the method based on the Reading Pressure Technique (Mauricio *et al.,* 1999). Gas pressure was converted to gas volume using a predetermined regression equation. In the first gas production trial, the gas production included gas produced by the energy sources, while in the second gas production trial, the energy source gas production was deducted from the total gas production to determine the effect of energy source on gas production of respective forage substrates *per se.* Data were fitted to two non-linear models adapted from Ørskov and McDonald (1979). Significant forage x energy interactions were observed for the non-linear parameter gas production (b) in Model 1 and for b and lag phase (L) in Model 2 in both trials. In the first gas production trial, the higher fermentability of the energy sources supplemented to forage substrates, increased b (Model 1 & 2) of the LUC and WS. The gas production rate was affected in different ways for different forages, with the most noticeable effect on WS when it was supplemented with energy sources. All the energy sources increased c of WS irrespective of the model used. Energy sources had no effect on the L of LUC, OH or RYE, but decreased the L of WS and KIK. In the second trial, maize meal had no effect on b for any of the forages (Model 1 & 2), while molasses (Model 1 & 2) decreased b for all forage substrates, and citrus pulp (Model 1 & 2) decreased b of OH and RYE, to lower values than those of the control treatments. Gas production rate was not affected by molasses for any of the forage substrates, while citrus pulp (Model 1 & 2) increased c of OH and maize meal increased c of OH and KIK. Lag phase was only affected by energy sources in WS and KIK, where all the energy sources had lower L values than the control treatment. It was concluded that forage fermentability is affected differently by different energy sources. These observations may have important implications, in practice, on rumen health and milk production, and the data obtained can potentially be used as guidelines in feed formulations.

In the second study, *in vitro* digestibility trials were undertaken to determine the effect of sugar (molasses and sucrose), starch (maize meal and maize starch) and pectin (citrus pulp and citrus pectin) on neutral detergent fibre (NDF) and dry matter (DM) degradability of forages. Forage substrates used included wheat straw, oat hay, lucerne hay, ryegrass and kikuyu grass. Rumen fluid was collected from two lactating Holstein cows receiving a diet consisting of oat hay, wheat straw and a concentrate mix. *In vitro* degradability was done with an ANKOM Daisy II incubator and forage substrates were incubated with or without the respective energy sources for 24, 48 and 72 hours. The substrates were incubated in 1076 ml buffered medium, 54 ml of reducing solution and 270 ml rumen fluid. The residues were washed, dried and analyzed for NDF. In the study with the applied energy sources (molasses, maize meal and citrus pulp) there were a forage x energy source interactions. Supplementation with the applied energy sources all improved dry matter degradability (DMD) of forages (24 and 72 hours), when compared to the control treatment, except for RYE supplemented with maize meal and citrus pulp at 24 hours. Molasses seemed to have had the biggest effect on DMD in all forage substrates. Supplementation with maize meal had no effect on neutral detergent fibre degradability (NDFD) of any forage substrate, except for an improvement in NDFD of LUC at 72 hours. Molasses improved NDFD of LUC at 24h, but had no effect on the other forage substrates. Citrus pulp improved NDFD of OH (72 hours), as well as LUC and WS (24 and 72 hours). It is postulated that the NDF of the energy sources was more digestible than that of the respective forages, and that the improved NDFD values could be ascribed to the contribution of the energy source NDFD. Overall, pasture grasses had a higher NDFD than the hays and straw, and appear to be more readily fermentable by

rumen microbes than the low quality hays and straw explaining the higher NDFD. In the study involving the purified energy sources (sucrose, maize starch and citrus pectin), forage x energy source interactions were observed. In general, supplementation with these energy sources improved DMD at 24 and 72 hours except for RYE and KIK (72 hours). Pasture grasses (RYE and KIK) had a higher NDFD than LUC, OH and WS. At 72 hours, NDFD was 37.1% for LUC, 42.5% for OH and 40.3% for WS, compared to 70.5% for KIK and 64.9% for RYE. A possible explanation is that KIK and RYE samples came from freshly cut material, harvested after a 28d re-growth period. In general, sucrose (24 and 72 hours) and citrus pectin (72 hours) had no effect on NDFD of forage substrates. However, supplementing oat hay (24 hours) with starch and citrus pectin, and wheat straw (24 and 72 hours) with starch lowered NDFD, when compared to the control treatment. It is hypothesized that microbes fermented the easily fermentable energy sources first, before attacking forage NDF. The study suggested that forage NDFD values are not fixed, and may be altered by type of energy supplementation.

Uittreksel

Titel: Die invloed van stysel, suiker en pektien as mikrobiese energiebronne op *in vitro* ruvoer-fermentasiekinetika

Naam: Marcia Malan

Studieleier: Prof. C.W. Cruywagen

Instansie: Departement Veekundige Wetenskappe, Universiteit van Stellenbosch

Graad: MScAgric

Die meervoudige maagsisteem van herkouers stel hulle in staat om ruvoer meer effektief te benut as enkelmaagdiere. Ruvoere alleen bevat egter nie genoeg voedingstowwe om die behoeftes van hoog-produserende melkbeeste te bevredig nie. Ruvoere is ryk aan vesel en hul voedingstofbeskikbaarheid word bepaal deur die graad van selwand degradeerbaarheid. 'n Verhoging in ruvoerfermentasie sal energie-inname verhoog en gevolglik ook melkproduksie en prestasie. Dit is dus belangrik om maniere te vind om ruvoerdegradeerbaarheid en -verbruik in die rumen te verbeter.

Die gebruik van verskillende nie-vesel koolhidraat (NFC) bronne het verskillende uitwerkings op die prestasie van diere. Energie-aanvullings soos suiker, stysel en pektien tot ruvoer-gebasseerde diëte, beïnvloed prestasiemaatstawwe soos melkproduksie, melksamestelling en droëmateriaalinname (DMI) op verskillende maniere.

Hierdie tesis lewer verslag oor twee studies waar die invloed van energie-aanvullings op ruvoerfermentasie en verteringsmaatstawwe ondersoek is. In die eerste studie is 'n *in vitro* gasproduksieprotokol gebruik om die invloed van suiker (melasse), stysel (mieliemeel) en pektien (sitruspulp) op totale gasproduksie (b) en tempo van gasproduksie (c) van verskillende ruvoersubstrate te bepaal. Ruvoersubstrate wat gebruik is, was koringstrooi (WS), hawerhooi (OH), lusernhooi (LUC), raaigras (RYE) en kikuyugras (KIK). Die drie energiebronne, sowel as 'n kontrole (geen energiebron), is *in vitro* geïnkubeer saam met elk van die genoemde ruvoere. Rumenvloeistof is verkry van twee lakterende Holsteinkoeie, wat 'n dieet ontvang het bestaande uit hawerhooi, koringstrooi en 'n kragvoermengsel. Ruvoere is alleen en/of in kombinasie met melasse (0.1412 g DM), sitruspulp (0.1425 g DM) of mieliemeel (0.125 g DM) in glasbottels afgeweeg en vir 72 uur geïnkubeer. Die massas van die energiebronne is op 'n energie-ekwivalente basis bereken. Leë bottels wat geen substraat bevat het nie, is ingesluit om te korrigeer vir gasproduksie afkomstig vanaf rumenvloeistof alleen. Substrate is in 40 ml van 'n buffermedium, 2 ml reduserende oplossing en 10ml rumenvloeistof geïnkubeer. Gasdruk is elke vyf minute outomaties aangeteken deur gebruik te maak van 'n drukmetersisteem en die metode is gebasseer op die Reading gasdruktegniek. Gasdruk is omgeskakel na

gasvolume deur gebruik te maak van ‘n voorafbepaalde regressievergelyking. In die eerste proef het totale gasproduksie die gas wat deur die onderskeie energiebronne geproduseer is, ingesluit. In die tweede proef is gasproduksie afkomstig van die energiebronne afgetrek van totale gasproduksie, om sodoende die invloed van die energiebronne *per se* op die gasproduksie van die onderskeie ruvoersubstrate, te bepaal. Data is met behulp van twee nie-liniëre modelle gepas. Betekenisvolle ruvoer x energie-interaksies is in albei proewe waargeneem vir die nie-liniëre parameter b (gasproduksie) in Model 1, en vir b en L (sloerfase) in Model 2. In die eerste proef het die energiebronne se hoë fermentasie gelei to ‘n verhoging in b (Model 1 & 2) van LUC en WS. Energie-aanvullings het die c-waarde van die onderskeie ruvoere verskillend beïnvloed, met WS wat die mees opvallende effek gehad het. Al die energiebronne het die c-waarde van WS verhoog, ongeag watter model gebruik is. Energiebronne het geen invloed op die L-waarde van LUC, OH of RYE gehad nie, maar het wel die L-waarde van WS en KIK verlaag. In die tweede proef het mieliemeel geen invloed op die b-waarde van enige van die ruvoere gehad nie (Model 1 & 2), terwyl melasse (Model 1 & 2) die b-waarde van alle ruvoere verlaag het, en sitruspulp (Model 1 & 2) OH en RYE se b waardes verlaag het tot laer as die kontroles. Melasse het geen invloed op die c-waarde van die onderskeie ruvoersubstrate gehad nie, terwyl sitruspulp (Model 1 & 2) die c-waarde van OH, en mieliemeel die c-waarde van OH en KIK, verhoog het. Energiebronne het slegs ‘n invloed op die sloerfase in WS en KIK gehad, waar dit L verlaag het tot laer waardes as dié van die kontroles. Daar is gevind dat ruvoer-fermenteerbaarheid verskillend beïnvloed word deur verskillende energiebronne. Bogenoemde resultate kan in die praktyk betekenisvolle invloede hê op rumengesondheid en melkproduksie en die data wat verkry is, kan potensieël gebruik word as riglyne in voerformulerings.

In die tweede studie is *in vitro* verteerbaarheidsproewe gedoen om die effek van suiker (molasse en sukrose), stysel (mieliemeel en mieliestysel) en pektien (sitruspulp en sitrus-pektien) op neutraal-onoplosbare vesel (NDF) en droë materiaal (DM) degradeerbaarheid van ruvoere, te bepaal. Ruvoersubstrate wat gebruik is, was WS, OH, LUC, RYE en KIK. Rumen vloeistof is verkry van twee lakterende Holstein koeie, wat ‘n dieet ontvang het bestaande uit hawerhooi, koringstrooi en ‘n konsentraat mengsel. Die *in vitro* degradeerbaarheidsproef is gedoen met ‘n ANKOM Daisy II inkubator. Ruvoersubstrate is geïnkubeer met of sonder die onderskeie energiebronne vir 24, 48 en 72 uur. Die substrate is geïnkubeer in 1076 ml buffer medium, 54 ml reduserende oplossing en 270 ml rumen vloeistof. Residue is gewas, gedroog en geanaliseer vir NDF. In die proef met toegepaste energiebronne (molasse, mieliemeel en sitruspulp), was daar ruvoer x energiebron interaksies. Toegepaste energiebron aanvullings het almal DMD van ruvoersubstrate (24 en 72 uur) verbeter, uitsluitend vir RYE wat aangevul is met mieliemeel (24 uur) en sitruspulp (24 uur). Van al die ruvoersubstrate het molasse die grootste effek gehad op DMD. Mieliemeel aanvullings het geen effek gehad op neutraal-onoplosbare vesel degradeerbaarheid (NDFD) van ruvoersubstrate nie, behalwe vir ‘n verbetering in NDFD van LUC by 72 uur. Molasse het NDFD van lucern by 24 uur verbeter, maar geen effek gehad op ander ruvoersubstrate nie. Sitruspulp het NDFD van OH (72 uur), asook LUC en WS (24 & 72 uur) verbeter. Daar word beweer dat die NDF van energiebronne meer verteerbaar is as die van ruvoersubstrate, en dat die verbetering in NDFD waardes toegeskryf kan word aan die bydraes van energiebronne se NDFD. Weidingsgrasse (RYE & KIK) het oor die algemeen ‘n hoër NDFD as hooie en strooi gehad. Rumen mikrobes blyk ook om dié grasse vinniger te verteer as lae kwaliteit hooie en strooi, wat gevolglik die hoër NDFD verduidelik. In die proef met suiwer energiebronne (sukrose, mieliestysel en sitrus-pektien) is ruvoer x energiebron interaksies waargeneem.

Energiebronaanvullings het DMD by 24 en 72 uur verbeter, buiten vir RYE en KIK (72 uur). Weidingsgrasse het hoër NDFD as LUC, OH en WS. By 72 uur was die NDFD van LUC 37.1%, OH 42.5%, WS 40.3%, in vergelyking met 70.5% vir KIK en 64.9% vir RYE. ‘n Moontlike verklaring vir die hoër NDFD van KIK en RYE, is omdat dit vars gesnyde material is, geoes na slegs 28 dae hergroei. Oor die algemeen het sukrose (24 & 72 uur) en sitrus-pektien (72 uur) geen effek gehad op NDFD van ruvoersubstrate nie, terwyl stysel en pektien aanvullings tot OH (24 uur), en stysel aanvullings tot WS (24 & 72 uur) NDFD verlaag het. Daar word hipotetieseer dat mikrobes eers die vinnig fermenteerbare energiebronne fermenteer, voordat hulle ruvoer NDF aanval. Hierdie studie beweer dat ruvoer NDFD waardes nie vas is nie, en dat dié waardes beïnvloed mag word deur energiebron aanvullings.

Acknowledgements

I wish to thank the following people and organizations:

- Prof. C.W. Cruywagen for his support and guidance
- Dr. Nherera for her help and support
- The Hennie Steenberg Trust Fund for funding for the study
- The Western Cape Department of Agriculture (Elsenburg) who made cannulated Holstein cows available for the collection of rumen fluid
- Academic and technical staff at the Department of Animal Sciences, Stellenbosch University, for providing support where necessary and an ideal working environment
- Fellow students who provided me with help and support throughout
- My parents for their motivation and support
- Al my friends for their motivation, support and help

Table of Contents

List of Tables

Deleted: 8

Deleted: 9

Deleted: 11

Deleted: 6

Deleted: 7

Deleted: 1

Deleted: 8

Deleted: 7

Deleted: 7

Deleted: ¶

Deleted: 9

Deleted: 30

Deleted: 30

Deleted: ¶
35

Deleted: 41

Deleted: 41

Deleted: 43

Deleted: ¶
¶
48

Deleted: 49

Deleted: 57

Deleted: 57

Deleted:

Deleted: 58

Deleted: ¶
59

Deleted: 60

Deleted: ¶
60

Deleted: 61

Deleted: 65

Deleted: 67

Deleted: 9

Deleted: 70

List of Figures

11 Deleted: ¶
Deleted: 2

14 Deleted: ¶
15

34 Deleted: 6

35 Deleted: 7

36 Deleted: 8

36 Deleted: 9

37 Deleted: 9

37 Deleted: 40

42 Deleted: 4

42 Deleted: 5

43 Deleted: 6

44 Deleted: 6

44 Deleted: 7

45 Deleted: 7

List of Abbreviations

ADF	Acid detergent fibre
ADL	Acid detergent lignin
b	Gas production
c	Gas production rate
$C_3H_7NO_2 \cdot HCL$	Cysteine hydrochloride
cp	Citrus pulp
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
KIK	Kikuyu grass
KOH	Potassium hydroxide
L	Lag phase
LUC	lucerne hay
mm	Maize meal
mol	Molasses
MP	Microbial protein
$Na_2S \cdot 9H_2O$	Sodium sulfide nonahydrate
ND	Neutral detergent
NDF	Neutral detergent fibre
NDSF	Neutral detergent-soluble fibre
NFC	Non-fibre carbohydrates
NH_3N	Ammonia nitrogen
NPN	Non-protein nitrogen
NSC	Non-structural carbohydrates
OH	Oat hay
OM	Organic matter
pef	Physical effectiveness factor
peNDF	Physical effective neutral detergent fibre
RDP	Rumen degradable protein
RPT	Reading pressure technique
RYE	Ryegrass
VFA	Volatile fatty acids
WS	Wheat straw

Chapter 1

INTRODUCTION

The compound stomach system of ruminants is adapted to roughage based diets, mainly grass (Cherney, 1998). Diets of grass and other fibrous feeds, however, do not meet the energy requirements of high producing dairy cows (Schwarz *et al.*, 1995). Fibre is low in energy and a large consumption thereof results in rumen fill, thus limiting feed intake (Allen & Oba, 2000). Concentrate feeds, such as non-fibre carbohydrates (NFC), provide lactating dairy cows with energy needed to improve performance and efficiency of production (Henning, 2004). Balanced rations consisting of forages and concentrate feeds ensure optimal production and rumen health. For dairy cattle, forages should comprise at least 40% of the diet and NFC should constitute between 35 and 42% of the diet (NRC, 2001). Non-fibre carbohydrates such as sugars, starch and pectin are critical in meeting energy requirements for growth and production (Roche & Dalley, 1996).

The symbiotic relationship between rumen micro-organisms and the host animal is an essential component in nutrient supply (Van Saun, 1998). Ruminant rations should provide the rumen micro-organisms with sufficient nutrients and an optimal environment for growth (Ishler *et al.*, 1996). According to the NRC (2001), the most important nutrients for optimal microbial growth are protein and carbohydrates. Microbial fermentation and digestion of carbohydrates and protein provide ruminants with volatile fatty acids (VFA) and microbial protein (MP). The animal uses the VFA as energy and the MP for protein synthesis (Van Saun, 1998).

Energy shortages affect lactating cows, especially during the first three weeks after calving (Hutjens, 1998). During this time dry matter intake (DMI) is low and milk production is high, resulting in a negative energy balance. When formulating diets for lactating dairy cows, it is important to consider the total NFC fraction, which primarily comprise of sugars, starch and pectins (Larson, 2003). The NFC ferment rapidly in the rumen to VFA (Holtshausen, 2004). Batajoo & Shaver (1994) reported that cows receiving diets with more than 30% NFC produced more than 40 kg of milk/day. However, they found no milk yield benefits by increasing the NFC beyond 36%. Molasses is a common energy supplement used in dairy rations (Holtshausen, 2004). In addition to this, molasses also reduce dustiness and increase palatibility and moisture content of diets (De Ondarza, 2000). Other energy supplements include soybean hulls, sugar beet pulp and citrus pulp. Leiva *et al*. (2000) showed that substituting diets that contain 20.5% citrus pulp (pectin) for diets containing 19.5% maize meal (starch), increased milk yield. Solomon *et al*. (2000), however, reported that substituting starch-rich diets with pectin–rich diets had no effect on milk yield.

Energy sources such as sugar, starch and pectin are frequently used as supplements to forage in ruminant diets, in order to meet the energy requirements for growth and production. However, there is a lack of information on the magnitude of the relationship between different carbohydrate sources and rumen neutral detergent fibre fermentation kinetics.

The objectives of this study were to determine the impact of three energy sources, *viz.* maize meal, molasses and citrus pulp on total gas production, rate of gas production, dry matter (DM) degradability, and NDF degradability of different forage substrates. Forages commonly used in dairy cow diets were chosen as fermentation substrates. These were wheat straw (*Triticum aestivum*), oat hay (*Avena sativa)*, lucerne hay (*Medicago sativa*), kikuyu (*Pennisetum clandestinum*) and ryegrass (*Lolium multiflorum*).

1.1. References

Allen, M. & Oba, M., 2000. Getting more milk from forages. Michigan Dairy Review 5(4), Department of Animal Science, Michigan State University.
Available at:
http://www.admani.com/alliancedairy/TechBulletins/Non%20Structural%20Carbohydrate%20Nutrition.htm
(Accessed 5 August 2008)

Batajoo, K.K. & Shaver, R.D., 1994. Impact of nonfiber carbohydrate on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 77, 1580 - 1588.

Cherney, D.J.R., 1998. Forages for dairy cattle: Economical alternatives to alfalfa, grass, and corn. In: Proceedings of the 1998 Tri-State Dairy Nutrition Conference, April 21 - 22, Fort Wayne, Indiana, USA. pp. 35 - 50.

De Ondarza, M.B., 2000. Non-fibre carbohydrates.

Available at: http://www.milkproduction.com/Library/Articles/Non_Fibre_Carbohydrates.htm
(Accessed 5 August 2008)

Henning, P., 2004. Acidosis in high producing ruminants - myth or menace? Animal Feed Manufacturers Association (AFMA), South Africa, pp. 1 - 9.
Available at: http://www.engormix.com/e_articles_view.asp?art=529&AREA=GDC
(Accessed 5 Augustus 2008)

Holtshausen, L., 2004. Effect of nonfibre carbohydrates on product yield and fibre digestion in fermentations with mixed ruminal microbes. PhD thesis, University of Florida, Gainesville, Florida, USA. pp. 1 - 33.

Hutjens, M.F., 1998. Practical approaches to feeding the high producing cow. Illini DairyNet. University of Illinois Extension.
Available at: http://www.livestocktrail.uiuc.edu/dairynet/paperDisplay.cfm?ContentID=247
(Accessed 5 Augustus 2008)

Ishler, V., Heinrichs, J. & Varga, G., 1996. From feed to milk: Understanding rumen function. Pennsylvania State University Extension Circular 422.

Available at: http://animsci.agrenv.mcgill.ca/courses/450/extra/feed_to_milk/concepts.html
(Accessed 25 August 2008)

Larson, C.C., 2003. The effect of nonfiber carbohydrate source and protein degradability on lactation performance of holstein cows. MSc (Agric) thesis, University of Florida. Gainesville, Florida, USA. pp. 1 - 4.

Leiva, E., Hall, M.B. & Van Horn, H.H., 2000. Performance of dairy cattle fed citrus pulp or corn products as sources of neutral detergent-soluble carbohydrates. J. Dairy Sci. 83, 2866 - 2875.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. (7th Rev. Ed.). National Academy Press, Washington, D.C., USA. pp. 34 - 35.

Roche, J. & Dalley, D., 1996. Nutrition and milk composition. Agriculture Notes. State of Victoria. Department of Primary Industries, pp. 1 - 3.
Available at:
http://www.dpi.vic.gov.au/dpi/nreninf.nsf/9e58661e880ba9e44a256c640023eb2e/036a3ac34d507323ca257181001f0359/$FILE/AG0534.pdf
(Accessed 1 October 2008)

Schwarz, F.J., Haffner, J. & Krichgessner, M., 1995. Supplementation of zero-grazed dairy cows with molassed sugar beet pulp, maize or cereal-rich concentrate. Anim. Feed Sci. Technol. 54, 237 - 248.

Solomon, R., Chase, L.E., Ben-Ghedalia, D. & Bauman, D.E., 2000. The effect of nonstructural carbohydrate and addition of full fat soybeans on the concentration of conjugated linoleic acid in milk fat of dairy cows. J. Dairy Sci. 83, 1322 - 1329.

Van Saun, R.J., 1998. Beef cattle nutrition: Feeding for two (How to properly feed the cow and her rumen). In: Cow-calf management guide-cattle producer's library (2nd Ed.). Cooperative Extension Service Publications, Agricultural Publications, University of Idaho, Moscow, Idaho, USA. pp. 172 - 180.

Chapter 2

LITERATURE REVIEW

2.1. Introduction

With the increase in the cost of feed, medicine, labour, fuel and other production essentials, it is vital for any agricultural enterprise, specifically the traditional 'farm', to be operated like a business. With the profit-making goal in mind, the single most important facet of a modern dairy-operation is its feeding program. An efficient and successful feeding program will not only maximize the animal's production, but will also cut costs in other areas of the operation by increasing animal health, productive life-expectancy and reducing labour costs. A feeding program that achieve these goals will ultimately make the agricultural enterprise more economically competitive.

Dairy cattle require specific amounts of nutrients to support various levels of performance. Feeding high levels of concentrates (especially non-fibrous carbohydrates) to high producing dairy cows, is common in all intensive production systems around the world. The problem with high levels of concentrates in dairy cow diets is the risk of these diets causing digestive disturbances. The aim of diet formulation and thus nutritional management for intensive production systems must be to maximize productivity and overall efficiency, without enhancing digestive disturbances such as acidosis (Henning, 2004). A successful feeding program will meet the cow's nutritive needs for high production, minimize weight loss (during early lactation), prevent digestive upsets and maintain ruminal and animal health.

In order to achieve full genetic potential for high milk production, it is of the utmost importance to keep the rumens of dairy cows in a healthy state. The rumen is home to a wide diversity of micro-organisms (including bacteria, protozoa and fungi), collectively utilizing the extensive variety of feeds, which make up dairy cow diets (Kamra, 2005). Forages are the main component of dairy cow diets. Forages alone, however, do not meet the energy requirements of high producing dairy cows (Schwarz *et al.*, 1995). Supplementing dairy cow diets with concentrate feeds provide high milk producing cows with energy needed to improve efficiency of production and performance (Henning, 2004). Carbohydrates are the largest nutrient component of dairy diets and the most important source of energy for rumen micro-organisms. Carbohydrates, important for growth, reproduction, milk production and rumen health, make up roughly 70% of dry matter (DM) in dairy diets, making it the 'heart' of dairy diets (Mertens, 1997). Carbohydrates (fibre, starch and sugar) are degraded in the rumen to simple sugars, and then fermented into volatile fatty acids (VFA) by rumen bacteria, supplying up to 80% of the animal's energy requirements.

2.2. Non-fibre carbohydrates and non-structural carbohydrates

The three main components of the carbohydrate fraction of feeds referred to as non-fibre carbohydrates (NFC) are starch, NDSF (neutral detergent-soluble fibre) and sugars. The NFC fraction of feedstuffs is estimated from the following calculation as proposed by Holtshausen (2004):

100% - crude protein% - ether extract% - ash% - neutral detergent fibre% + neutral detergent insoluble crude protein%

The fraction derived by the above calculation has at times been used interchangeably for the terms NFC and non-structural carbohydrates (NSC). Non-structural carbohydrates, however, refer to plant cell contents and include mono- and oligosaccharides, organic acids (which are not carbohydrates), starch and fructans. Non-fibre carbohydrates include all of the above substances as well as soluble fibre (pectic substances, ß-glucans and galactans). Thus, NFC includes non-structural and structural carbohydrates, as well as non-fibrous and fibrous carbohydrates (Holtshausen, 2004).

In the interest of clarity, I will not use the terms NSC and NFC interchangeably. I will use NFC exclusively, as its meaning is more complete in the context of this thesis.

2.3. Rumen microbiology

All living organisms require some essential nutrients to sustain metabolic processes and to maintain a healthy state. These essential nutrients include water, protein, minerals, vitamins and essential energy. The difference between the cow itself and the micro-organisms living within its rumen is defined by the source of their respective nutrients (Van Saun, 1993).

Feeding dairy cattle nutritional balanced diets ensures healthy rumens that maximize microbial production and growth. Ruminal pH is the main variable influencing the microbial population and thus the overall VFA production (major energy source to animal). Diets containing too much NFC may cause the ruminal pH to decrease below 6. This low rumen pH leads to a reduction in cellulolytic organisms and an increase in propionate producing micro-organisms, in turn leading to a low acetate-propionate ratio. This in turn results in low milk fat percentages (Ishler *et al.,* 1996).

Maintaining a healthy rumen microbial population is an essential function of any feeding program. Carbon skeletons and energy are used by rumen micro-organisms for protein synthesis. Ruminant systems are sometimes based on digestible organic or fermentable matter, even though rumen micro-organisms are able to grow and develop on only secondary carbohydrate products. Rumen bacteria have specific maintenance requirements for growth and development. Both bacterial growth rate and fractional degradation rate of carbohydrates determine bacterial yield (Nocek & Tamminga, 1991).

2.4. Physical effective fibre and particle size

Preventing ruminal acidosis requires chemical and physical considerations of the diet, as well as a well-organized feed and herd management system (Hall, 2002). The physical form of the diet affects the nutritive value of the feed as well as the chewing activity, dry matter intake, rumen function and milk production of the animal.

Particle size plays a critical role in the extent to which rumen micro-organisms can carry out their digestive functions. Grinding or chopping forages does not change the forage composition, it only reduces the particle size. Reduced particle size increases dry matter intake and the rumen turnover rate, resulting in a reduced time period within which rumen micro-organisms can digest fibre. Reduced particle size also reduces the time spent on rumination, thus leading to less mucus production and a subsequent decrease in rumen pH. Low rumen pH leads to an increase in propionic acid production and tend to change milk components by lowering milk fat percentages and increasing milk protein percentages. Chopping and grinding of concentrates increase the starch exposure to rumen microbial digestion, resulting in increased degradation. Processing methods such as pelleting, steam rolling, or grinding of concentrates alter the structure of starch by increasing its availability for fermentation in the rumen. This increase in starch availability can be either favorable by boosting rumen microbial growth or harmful by enhancing the risk of rumen acidosis (Van der Merwe & Smith, 1991).

As with particle length, fibre content of the diet plays an important role in maintaining rumen functions. Fibre ensures sufficient amounts of carbohydrates to slow down the rate of digestion and prevent rumen acidity. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) are the most important fibre fractions in ration formulation. Effective fibre is needed in dairy diets to form a ruminal mat and slow down carbohydrate availability, thereby preventing rumen acidosis (Ishler *et al.,* 1996). Balancing the dairy ration for NDF and non-fibre carbohydrates (NFC) fractions is very important in controlling the rumen pH. Buffers are also commonly used for controlling pH.

Physical effective fibre (peNDF) relates the physical properties of a feed (by measuring particle size and chewing activity) to rumen pH. The peNDF of a feed is the product of the feed's physical effectiveness factor (pef) and the feed's NDF. By definition, pef varies between 0 (if NDF if is not successful in stimulating chewing activity) and 1 (if NDF is successful in encouraging chewing activity) (Mertens, 1997).

It is very important to always balance the peNDF of the dairy cow diet with dietary fermentability. Physical effective fibre is that fraction of fibre that promotes the chewing activity. Thus, when feeding lactating cows, it is very important to add adequate amounts of peNDF. Optimal inclusion of peNDF will ensure that the cow chews her cud well enough, and in the process secrete enough saliva that helps to control rumen pH. Ruminal pH is primarily determined by the balance between the quantity of fermentation acid produced, and the buffer secretion during chewing (Allen, 1997).

2.5. Forage classification

Good forage sources are the foundation of efficient dairy production (Morrison, 1959). A large variety of feeds can be defined as forages (Baloyi, 2008), as listed in Table 2.1. By definition, forages are the edible fractions of plants, other than grain, that can be harvested for feeding or used as feed for grazing animals (Forage & Grazing Terminology Committee, 1991). The definition also states that feedstuff must contain 35% or more NDF to be classified as forage (Zinn & Ware, 2007).

It is of utmost importance to remember that a high producing dairy cow's digestible nutrient and net energy requirements cannot be met by forage alone. Generally, dairy cows are fed good quality forages and then supplemented with additional grains or other concentrates in order to meet their requirements.

Table 2.1 Feed types that fall within the definition of forage (adapted from Wilkens, 2000).

Forage	Feed types
Herbage	Leaves, roots of non-woody species, stems, sown and permanent grasslands, crops that may be cut or grazed
Hay	Grasses and legumes that have been cut, dried and stored for use as animal feed
Silage	Fermented high moisture fodder
Browse	Leaves, bud and twigs of woody species
Straw	Dry stalk of cereal plant after the grain or seed has been removed

2.5.1. Factors influencing forage nutritive value

Chemical composition, digestibility and the physical characteristics of the digested feed determines the nutritive value of forage (Goosen, 2004). Forages between and within species differ significantly in composition and nutritive value, as indicated in Table 2.2.

Table 2.2 Energy (MJ/kg DM) and protein (g/kg DM) content of different classes of forages (Wilkens, 2000).

Forage class	**Metabolizable energy**	**Crude protein**
	MJ/kg DM	**g/kg DM**
Temperate grasses, hays and silages	7.0-13.0	60-250
Tropical grasses	5.0-11.0	20-200
Maize silage	10.0-12.0	60-120
Cereal straw	5.0-8.0	20-40
Root crops	11.0-14.0	40-130
Kale and rape	9.0-12.0	140-220

Age and maturity, soil fertility and environmental conditions are the primary factors influencing the nutritive value of forages. Herbage maturity has the largest influence on forage nutritive value (Buxton & Mertens, 1995). Mature forages have higher lignin and cell wall contents that limit fibre utilization due to the rate and degree of plant cell hydrolysis (Van Soest, 1994).

2.5.1.1. *Age and maturity*

Young plants are tender with less structural carbohydrates (hemicellulose and cellulose) and lignin compared to mature plants (McDonald *et al.*, 2002). Lignin is indigestible, explaining the higher digestibility in younger plants. As plants mature the stems and leaves become lignified, decreasing the nutritive value of the plant due to the lower digestibility of nutrients enclosed in the cell walls (Morrison, 1959). Leaves have lower cell wall content than stems. As the plant matures there is an increase in the proportion of stems compared to leaves, thus contributing to the lower digestibility of mature plants (Van Soest, 1994)

2.5.1.2. *Soil fertility and environment*

Environmental factors that affect forage quality the most are temperature, light, water and soil fertility (Van Soest, 1994). The mineral content in soil influences not only the crop yield, but also its composition. Fertilizers can have a great influence on the nutrient content of soils. Fertilized pastures grow better, are more palatable and have higher protein, vitamin and mineral contents than unfertilized pastures (Morrison, 1959).

2.6. Fibre

Fibre is composed of an indigestible fraction and several potentially digestible fractions that occupy space in the gastrointestinal tract of ruminants (Mertens, 1997). The primary components of fibre are cellulose, hemicellulose, and lignin. In the rumen, feed is digested through microbial fermentation and the physical breakdown of feed through rumination (Ishler & Varga, 2001). The type of diet fed influences and change bacteria population in the rumen in order to successfully digest the food used by the cows. The level to which fibre will digest depends on the particle size, rumen pH and fibre level in the diet.

2.7. Van Soest forage fraction analysis

Fibre, lignin and protein are the three most important chemical fractions determining nutrient supply and performance (Mould, 2003). According to Van Soest (1994) chemical analysis measures digestibility and intake using the statistical relationship between feed quality and the analyzed feed components. The proximate analysis divided feedstuff into six fractions, namely moisture, crude protein, ash, ether extract, nitrogen-free extract and crude fibre (Fisher *et al.*, 1995). Van Soest (1994) claimed that the proximate analysis had one serious error, namely that the proximate analysis divided carbohydrates into crude fibre and nitrogen-free extract. Van Soest then developed an analysis specifically for fibre-rich feeds that replaced the proximate analysis. The method of Van Soest predicts intake and the nutritive value of feedstuffs by determining the fibre fractions according to the degradability of fractions insoluble in neutral detergent, and fractions insoluble in acid detergent (Goosen, 2004). Acid detergent fibre determines the cellulose and lignin content and NDF the cellulose, hemicellulose and lignin. The difference between NDF and ADF gives the hemicellulose content (Knudsen, 2001). Table 2.3 gives an outlay of the components soluble and insoluble in NDF.

Table 2.3 Forage fraction classification using the method of Van Soest (Van Soest & Wine, 1967).

Fraction	Components
Cell contents (soluble in neutral detergent)	Lipids
	Sugar, organic acids
	Water-soluble matter
	Pectin, starch
	Non-protein nitrogen
	Soluble protein

Table 2.3(continue) Forage fraction classification using the method of Van Soest (Van Soest & Wine, 1967).

Cell wall contents (insoluble in neutral detergent)	
1. Soluble in acid detergent	Fibre-bound protein
	Hemicellulose
2. ADF	Cellulose
	Lignin
	Lignified N
	Silica

2.8. *In vitro* techniques for evaluating feed resources

In vitro methods used to evaluate feed resources are less time-consuming and less expensive than *in vivo* methods. The *in vitro* gas production procedure measures the amount of gas produced or collected, recording it manually (Theodorou *et al.*, 1994) or automatically (Pell & Schofield, 1993; Davies *et al.*, 2000). This procedure thus generates kinetic data rather than digested feed disappearance (Baloyi, 2008). Gas production gives a description of the microbial activity and how micro-organisms respond to a specific substrate, thereby giving a practical imitation of what happens in the rumen. Pell *et al.* (1998) used *in vitro* gas production to measure the rate and extent of fermentation, VFA production and microbial protein (MP) production. The biggest advantage of the gas production technique is that there is no need to terminate the gas production in order to measure the extent of digestion. The disadvantage of this technique, however, is the lack of uniformity in methodology and factors such as pH and temperature that may affect a feed's gas production (Getachew *et al.*, 1997). The traditional two-stage method (Tilley & Terry, 1963) involved an *in vitro* fermentation of forages in rumen fluid, followed by pepsin digestion. The disadvantage of this technique, however, is that it is an end-point measurement, thus giving no indication on forage digestion kinetics (Theodorou *et al.*, 1994). Goering & Van Soest (1970) modified the procedure to accurately estimate the true DM digestibility by treating the residue with a ND solution (Baloyi, 2008). The method of Goering and Van Soest, however, are also an end-point measurement, thus giving no indication on forage digestion kinetics (Theodorou *et al.*, 1994). ANKOM technology developed a technique that simplifies *in vitro* digestibility evaluations, using an insulated incubator (Daisy II incubator) (Baloyi, 2008). The ANKOM technique predict potential and true digestibility *in vitro* accurately, faster and with less labour.

2.9. Carbohydrates

Carbohydrates can be classified into two groups (structural carbohydrates and non-fibrous carbohydrates) based on their function in plants (see Figure 2.1). Structural carbohydrates, which are located in the plant's cell walls, are very fibrous and are digested slowly. Non-fibrous carbohydrates are located in the plant's

leaves and seeds. Non-fibrous carbohydrates are easily digested and include starches, sugars, fructans and organic acids (Ishler & Varga, 2001).

Acid detergent fibre and NDF are the most universal way of analyzing fibre. It is important to note that although pectins are part of the cell wall, they are grouped as non-structural carbohydrates. The reason for this is because pectin, other than hemicellulose, is entirely fermentable by rumen micro-organisms (Van der Merwe & Smith, 1991).

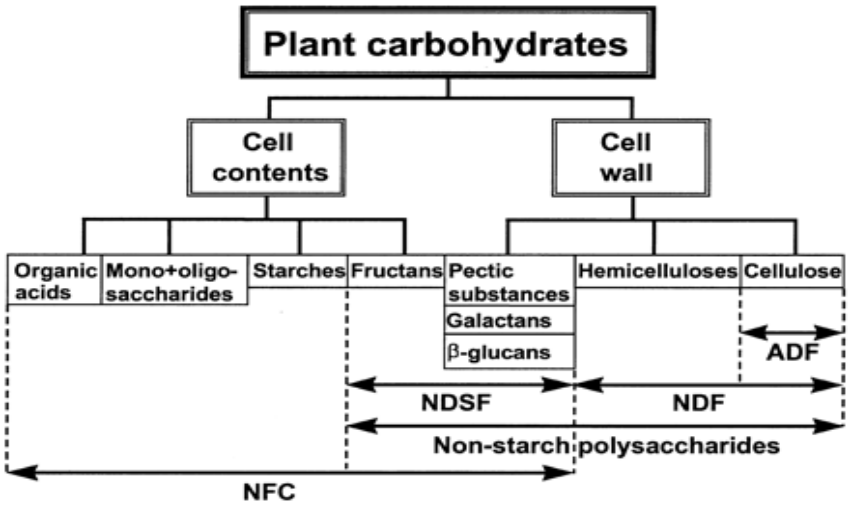

Figure 2.1 Structural and non-structural carbohydrates of plants where ADF = acid detergent fibre, ß-glucans = (1 → 3) (1 → 4)-ß-D-glucans, NDF = neutral detergent fibre, NDSF = neutral detergent-soluble fibre (includes all non-starch polysaccharides not present in NDF), NSC = non-NDF carbohydrates (Ishler & Varga, 2001).

2.9.1. Non-fibre carbohydrates / non-structural carbohydrates

Non-fibre carbohydrates are the major source of energy for high producing dairy cattle all around the world. Non-fibre carbohydrates are very palatable and easily digested, but fermentation varies with type of feed and means of processing. Increasing NFC in the diet fulfils the high energy demands of a lactating dairy cow, but at the expense of NDF (NRC, 2001).

The non-structural component of plants can be identified by two different methods: chemical analysis (which uses enzymes to determine the level of starch and sugar in the feed) or difference calculations (which use NDF, crude protein, fat and ash to estimate NFC) (Stokes, 1997). Russell *et al*. (1992) reported that greater amounts of NFC in dairy cow diets increase the production of MP. Thus, NFC in diets for lactating cows has the potential to increase MP synthesis, as well as the efficiency of ruminal undegradable protein utilization (Casper *et al.*, 1990). It must, however, be emphasized that MP yield differs with different NFC sources.

Different NFC sources require different inclusions of rumen degradable protein and rumen undegradable protein in order for an animal to reach optimal performance (Mertens *et al.*, 1994).

Milk production per cow is the major factor determining any dairy farm's profitability. The inclusion of NFC is a fashionable way to increase energy density and thus milk production of the dairy herd. Replacing part of the starch in the diet with sugar leads to higher fermentation rates and more MP. This might be due to the fact that sugar digests at a rate of 300% per hour, whereas starch digests at a rate of 6 – 60% per hour (Carver, 2007). Research has also shown that additional supplementary sugar in feed has the power to increase feed intake, milk yield and fat content of milk, due to better fibre digestion and production of MP in the rumen (Lykos *et al.*, 1997).

The importance of adequate amounts of NFC cannot be over emphasized. Feeding inadequate amounts of NFC reduces the energy available from propionic and lactic acid production, reduces MP synthesis and decrease fibre digestion. Overfeeding of NFC depress fibre digestion and acetic acid production (lowering milk fat percentages).

It is important to note that NFC and NSC is not the same in all feeds. The difference between these two is caused by the input of pectin and organic acids. Pectin is always included in NFC but not in NSC (NRC, 2001). Numerous research experiments investigated the effect of NFC on ruminal pH. Knowledge of the individual, as well as a combination of supplemented NFC fermentation characteristics, can be helpful in predicting an animal's performance (Holtshausen, 2004).

2.9.1.1. *Sugar*

Simple sugars are rapidly fermented in the rumen (at a rate of 300% per hour) and are composed of one or two units of sugar. Sugars commonly fed to dairy cows include sucrose, lactose and dextrose. Initially, sugar was used in diets to improve the palatability of the feed. Recently it was discovered that rumen micro-organisms respond to sugar by increasing their production of MP, leading to higher milk production. The addition of sugar to the feed also helps rumen micro-organisms capture and utilize diet nitrogen. Even though sugar has very advantageous effects on rumen micro-organisms and their actions, it is important not to add too much sugar in dairy cow diets, as it can cause ruminal acid-spikes resulting in acidosis (De Ondarza, 2000).

2.9.1.2. *Starch*

The NFC in most grain-based diets is made up of starch (24 – 28% of the total ration DM). Starch digestibility plays an important role in the milk production of dairy cows. Maize and barley (being cereal grains) provide most of the starch in a dairy cow's diet. Theoretically, starch is units of glucose bonded together. Depending on the starch source and method of processing the glucose units can be firmly bonded or weakly connected. This is the main reason why some starches ferment rapidly and others slowly in the

rumen of dairy cows. Ruminal digestion of starch can vary from 6 – 60% per hour depending on the starch source and processing method used. The goal of feeding starch is to achieve maximum total tract digestibility and maximum MP production, without causing ruminal health problems due to production of fermentation acids (De Ondarza, 2000).

2.9.1.3. *Pectin*

Pectin is one of the three most essential structural components in forages, and is found primarily in the intercellular layers of plant tissues. Pectin diminishes as the plant gets older. Most feeds consumed by dairy cows are low in pectin (2 – 3%), but several feeds may contain higher concentrations, such as citrus pulp (15%), beet pulp (15 – 20%), and lucerne (3 – 10%). Pectin contributes to the energy requirements of rumen micro-organisms (75 – 90% of pectin fermentation takes place in the rumen) (Allen, 2001).

Many of the species that break down pectin also digest plant components such as cellulose and hemicellulose. Pectin is extremely fermentable and highly digestible, but this does not appear to lower pH as is often seen with starch digestion. Due to this, feeds containing pectin are often supplemented into high concentrate dairy diets to avoid problems associated with rumen acidosis (Mohney, 2002).

A study done by Dehority (1969) found that a number of different rumen bacteria are capable of fermenting pectin (using it as a carbon source), e.g. *Butyrivibrio fibrisolvens, Prevotella ruminicola, Lachnospira multiparus, Treponema bryantii* and *Succinivibrio dextrinosolvens*. Later studies discovered that the products of the hydrolysis (by *Lachnospira multiparus bacterium*) of pectic material can be used by other ruminal micro-organisms such as *Selenomonas ruminantium, Fusobacterium sp., Eubacterium ruminantium* and *Succinivibrio dextrinosolvens* (Paggi *et al.*, 2005).

2.9.1.4. *Sugar vs. Starch*

Leiva *et al*. (2000) investigated the effect of two maize silage/lucerne hay-based diets on ruminal pH. The only difference between these two diets was that their NFC came from either starch (hominy) or sugars (dried citrus pulp). From this trial, it was concluded that the pH declined more rapidly on citrus diets (sugar) than on hominy (starch) diets and also reaching the lowest pH point faster (Figure 2.2).

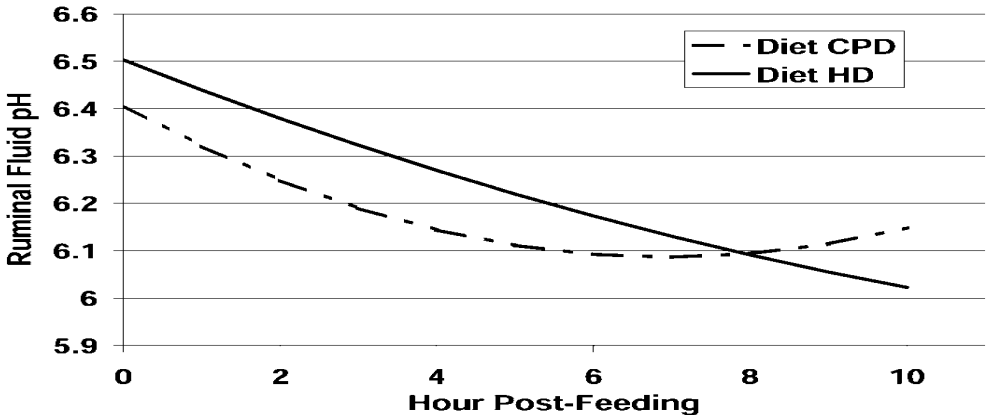

Figure 2.2 Ruminal pH results for citrus and hominy rations, where CPD = citrus pulp diet and HD = hominy diet (Leiva *et al.*, 2000).

From the studies done by Leiva *et al*. (2000) it was observed that starch has the best chance of increasing milk production. However, the problem with high starch diets is that they are likely to cause serious rumen acidosis. One way to ensure healthy rumens, as well as high milk production, is to use peNDF as a benchmark. The more peNDF a cow consumes the more starch may be included in the diet. Physical effective neutral detergent fibre thus lowers the risk of ruminal acidosis. The effect of supplementing sugar to forage based diets is shown in Table 2.4.

Table 2.4 The effect of sugar on dairy cattle performance.

Reference	Forage Source	Supplements	Intake, g DM / day	Animal Response
Chamberlain *et al.*, 1985	Grass	Sugar	907.2 g sugar	MP synthesis ↑
Huhtanen, 1988	Grass	Molasses	997.9 g molasses (499 g sugar)	OM digestion & MP production ↑
Nombekela & Murphy, 1995	Lucerne Haylage & Maize Silage	Sucrose	285.8 g sucrose	Milk yield (907.2 g) & DMI ↑

MP = microbial protein; OM = organic matter; DMI = dry matter intake.

2.10. Non-fibre carbohydrate digestibility

Non fibre carbohydrates are composed of starch and sugar. Starch digestibility has a major effect on the rumen. Starch fermentation varies with processing and type of grain fed. Processing such as grinding,

steaming and ensiling affects ruminal availability of starch. Processing mostly increases rate of fermentation and digestibility of starch. Soluble sugar ferments rapidly in the rumen and is readily available as energy sources for the animal (Ishler & Varga, 2001). Mature grains (maize or oats) usually contain a small amount of sugar, because most has been converted to storage polysaccharides. Forages (pasture or hay) usually contain a lot of sugars. The level of sugar in hay is depended on crop management. Byproducts (molasses, citrus pulp, and almond hulls) contain high levels of sugars. However, the variation in processing methods (as in the case of starch) and the type of material used can lead to large variation in sugar content (Hall, 2002).

One problem with diets high in NFC is the fact that it lowers the rumen pH, increasing the risk of acidosis. The main reason for this is NFC fast fermentability, especially if it replaces fibre in low fibre diets. Acidosis in turn affects ruminal digestion, intake, metabolism, milk fat production, milk production, as well as rumen and animal health. The NFC levels of various feed types are shown in Table 2.5.

Table 2.5 Non-fibre carbohydrate levels for various ration types (Ishler & Varga, 2001).

Typical NFC level	Typical feedstuffs
33 – 36%	Barley, oats, high moisture-, steam flaked- and finely ground grain predominate the concentrate portion of the diet.
37 – 39%	High quality hay crop forages predominates the ration; maize silage rations include non-forage fibre sources.
40 – 42%	Coarsely processed maize is used; diet has a high inclusion level of non-forage fibre sources.

2.11. Non-fibre carbohydrate fermentation and organic acid production

The rate and extent of carbohydrate fermentation determines the concentration of organic acids produced. Rumen micro-organisms digest simple and complex carbohydrates (fibre) by converting them into VFA (mainly acetic, propionic, and butyric acid). These VFA are the most important energy source for ruminants. Volatile fatty acids account for 60 – 70% of metabolizable energy supply in ruminants, making it of great importance in the production of milk by dairy cows. Reduction in fibre digestion leads to a reduction in ruminal pH. This is caused by rapid NFC fermentations leading to increased VFA production by rumen micro-organisms.

Feeding large amounts of forage produces greater amounts of acetic acid, whilst resulting in lesser amounts of propionic and butyric acid. On the other hand, feeding grain or other finely ground forages may lead to a reduction in acetic acid, while the amount of propionic acid may increase. The ratio of acetic to propionic acids imitates the rumen fermentation pattern. Under an optimal rumen fermentation environment the acetic to propionic ratio should be greater than 2.2:1. High planes of acetate can point to a low fermentable carbohydrate, high fibre ration. High planes of propionic acid, on the other hand, can point to reduced fibre digestion and acidosis (University of Minnesota, 1996).

A study done by Strobel & Russel (1986) found that pectin fermentation increased acetate concentrations further, compared to starch and sucrose. The study concluded that the increased acetate might ultimately contribute to increase precursors for fatty acid and milk fat synthesis in lactating dairy cows. Fermentation studies done with sucrose and starch, on the other hand, increased butyrate production (Hoover *et al*., 2006). Butyrate has shown to be an important precursor of energy supplied to skeletal and heart muscles (Holtshausen, 2004). Sugar ferments extremely fast in the rumen. Without linkages to other carbohydrates and due to the high solubility of sugars, there is little to impede microbial fermentation (Hall, 2002). Several studies reported that sugar has a much higher potential for lactate production, when compared to other NFC sources (for example starch) (Strobel & Russel, 1986; Heldt *et al.*, 1999).

2.12. Ruminal acidosis

Micro-organisms in the rumen obtain energy primarily from fermentation of carbohydrates. Acidosis occurs when the diet of ruminants is suddenly changed from a forage based diet to a predominant concentrate diet. The highest risks come from diets that are high in starch or fast fermentable carbohydrates and the effective fibre is below the recommended level or the particle size is too small. This leads to higher VFA production as well as very high glucose levels in the rumen. Subsequently, ruminal osmolarity increases leading to ruminal acidity. The increase in osmolarrity is due to the negative effect the high glucose level has on *Streptococcus bovis* and lactic acid-producing micro-organisms (Henning, 2004). Acidosis can be divided into sub-acute ruminal acidosis and acute acidosis as seen in Table 2.6.

Table 2.6 Comparison of acute and sub-acute acidosis (Henning, 2004).

Item	**Acidosis**	
	Acute	***Sub-Acute***
Clinical Signs	Present	Absent
Systemic Acidosis	Present	Absent
Mortality	Yes	No
Ruminal pH	< 5,0	5,0 -5,5

Table 2.6(continue) Comparison of acute and sub-acute acidosis (Henning, 2004).

Ruminal Acids:		
Lactic Acid	High (50-100mM)	Normal (0-5mM)
Volatile Fatty Acids	Below Normal (<100mM)	High (150-200mM)
Ruminal Bacteria:		
Lactic Acid Producers	Very High	Normal to Small Increase
Lactic acid Utilisers	Significant Reduction	Increase
Ruminal Protozoa	Absent or Reduced	Absent or Reduced

Indicators determining whether ruminal acidosis is accruing in the herd, include (Ishler & Varga, 2001):

Milk fat percentage (↓ milk fat – ↓ ruminal pH)
Chewing activity (↓ rumination – ↑ ruminal acidosis)
Laminitis (↑ laminitis – ↑ ruminal acidosis)
Strategies for avoiding acidosis:

Provide good quality total mixed rations.
Give small but frequent meals.
Avoid abrupt changes in diets.

2.13. Conclusion

Achieving maximum production and maintaining a healthy rumen ecosystem at the same time is a balancing act. A cow will attain more VFA when fermentation is maximized. These VFA are used as energy precursors and to synthesize MP. Increased fermentation, however, goes together with increased acid production and a lower rumen pH. Low rumen pH can lead to metabolic disorders due to impaired fibre digestion. Thus, by increasing the peNDF intake the risk of acidosis can be reduced effectively.

Non-fibre carbohydrates are the essential source of energy for high producing dairy cattle. One problem with diets high in NFC, however, is the fact that it lowers the rumen pH increasing the risk of acidosis. This is mainly due to the fast fermentability of NFC, especially if it replaces fibre in low fibre diets. Acidosis, in turn, affects ruminal digestion, intake, metabolism, milk fat production and milk production, as well as rumen and animal health.

A better understanding of the workings of the rumen as a whole ecosystem as well as ensuring optimal sugar, starch and peNDF in dairy cow ration, will enable farmers to maintain the fine balance between productivity and acidosis.

Whilst the goal of any modern agricultural enterprise is to maximize profits, special care should be taken not to increase short-term profits at the cost of long-term viability. A dairy cow's productivity can easily be increased, whilst the animal's health could suffer in the long term. In the modern scenario, the farm is as much a profit-seeking enterprise as any other business. With this in mind, the modern farmer in his capacity as a business manager should ensure the well-being and optimal performance of his assets.

2.14. References

Allen, M.S., 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80, 1447 - 1462.

Allen, M., 2001. Formulating lactating cow diets for carbohydrates**.** In: Proceedings of the 5th Western Dairy Management Conference, April 4 - 6, Las Vegas, Nevada, USA. pp. 79 - 86.

Baloyi, T.F., 2008. Effects of exogenous fibrolytic enzymes on *in vitro* fermentation kinetics of forage and mixed feed substrates, MSc (Agric) thesis, Stellenbosch University, Stellenbosch, South Africa. pp. 1 - 28.

Buxton, D.R. & Mertens, D.R., 1995. Quality-related characteristics of forage. In: Forages: An introduction to grassland agriculture (2nd Ed.). Eds. Barnes, R.F., Miller, D.A. and Nelson, C.J., Iowa State University Press, Ames, Iowa, USA. pp. 83 - 96.

Carver, L.A., 2007. Sugar aids lactating dairy cattle production. Feedstuffs 79(2), 1 - 3.

Casper, D.P., Schingoethe, D.J. & Esenbeisz, W.A., 1990. Response of early lactation dairy cows fed diets varying in source of nonstructural carbohydrate and crude protein. J. Dairy Sci. 73, 1039 - 1050.

Chamberlain, D.G., Thomas, P.C., Wilson, W., Newbold, C.J. & MacDonald J.C., 1985. The effects of carbohydrate supplements on ruminal concentrations of ammonia in animals given diets of grass silage. J. Agric. Sci. Cam. 104, 331 - 340.

Davies, Z.S., Mason, D., Brooks, A.E., Griffith, G.W., Merry, R.W. & Theodorou, M.K., 2000. An automated system for measuring gas production from forages inoculated with rumen fluid and its use in determining the effect of enzymes on grass silage. Anim. Feed Sci. Technol. 83, 205 - 221.

Dehority, B.A., 1969. Pectin-fermenting bacteria isolated from bovine rumen. J. Bacteriol. 99, 189 - 196.

De Ondarza, M.B., 2000. Non-fibre carbohydrates.
Available at: http://www.milkproduction.com/Library/Articles/Non_Fibre_Carbohydrates.htm
(Accessed 5 August 2008)

Fisher, D.S., Burns, J.C. & Moore, J.E., 1995. The nutritive evaluation of forage. In: Forages: An introduction to grassland agriculture (1st Ed.). Eds. Barnes, R.F., Miller, D.A. & Nelson, C.J., Iowa State University Press, Ames, Iowa, USA. pp. 105 - 115.

Forage & Grazing Terminology Committee, 1991. Vegetation terms. In: Terminology for grazing lands and grazing animals. Pocahontas Press Inc., Blacksburg, Virginia, USA.

Getachew, G., Makkar, H.P.S. & Becker, K., 1997. The *in vitro* gas coupled with ammonia measurements for evaluation of nitrogen digestibility in low quality roughages using incubation measurements of different measuring capacity. J. Sci. Food Agric.

Goering, H.K. & Van Soest, P.J., 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications). Agricultural Handbook Number 379. ARS-USDA, Washington, D.C., USA.

Goosen, L., 2004. The effect of an exogenous fibrolytic enzyme on forage digestibility parameters, MSc (Agric) thesis, Stellenbosch University, Stellenbosch, South Africa. pp. 1 - 27.

Hall, M.B., 2002. Working with sugars (and molasses). In: Proceedings of the 13th Annual Florida Ruminant Nutrition Symp., January 11 - 12, Gainesville, Florida, USA. pp. 146 - 158.

Heldt, J.S., Cochran, G.L., Stokka, G.L., Farmer, C.G., Mathis, C.P., Titgemeyer, E.C. & Nagaraja, T.G., 1999. Effects of different supplemental sugars and starch fed in combination with degradable intake protein on low-quality forage use by beef steers. J. Anim. Sci. 77, 2793 - 2802.

Henning, P., 2004. Acidosis in high-producing ruminants - myth or menace? Animal Feed Manufacturers Association (AFMA), South Africa, pp. 1 - 9.
Available at: http://www.engormix.com/e_articles_view.asp?art=529&AREA=GDC
(Accessed 5 Augustus 2008)

Holtshausen, L., 2004. Effect of nonfibre carbohydrates on product yield and fibre digestion in fermentations with mixed ruminal microbes. PhD thesis, University of Florida, Gainesville, Florida, USA. pp. 1 - 33.

Hoover, W.H., Tucker, C., Harris, J., Sniffen, C.F. & De Ondarza, M.B. 2006. Effects of nonstructural carbohydrate level and starch:sugar ratio on microbial metabolism in continuous culture of rumen contents. Anim. Feed Sci. Technol. 128(3 - 4), 307 - 319.

Huhtanen, P., 1988. The effects of barley, unmolassed sugar-beet pulp and molasses supplements on organic matter, nitrogen and fibre digestion in the rumen of cattle given a silage diet. Anim. Feed Sci. Technol. 20, 259.

Ishler, V., Heinrichs, J. & Varga, G., 1996. From feed to milk: Understanding rumen function. Pennsylvania State University Extension Circular 422.
Available at: http://animsci.agrenv.mcgill.ca/courses/450/extra/feed_to_milk/concepts.html
(Accessed 25 August 2008)

Ishler, V. & Varga, G., 2001. Carbohydrate nutrition for lactating dairy cattle. Pennsylvania State University, Code #: DAS 01 - 29 (online), pp. 1 - 11.
Available at: http://www.das.psu.edu/teamdiary
(Accessed 5 August 2008)

Kamra, D.N., 2005. Rumen microbial ecosystem. Current Science 89(1), 124 - 135.

Knudsen, K.E. 2001. The nutritional significance of 'dietary fibre' analysis. Anim. Feed Sci. Technol. 90, 3 - 20.

Leiva, E., Hall, M.B. & Van Horn, H.H., 2000. Performance of dairy cattle fed citrus pulp or corn products as sources of neutral detergent-soluble carbohydrates. J. Dairy Sci. 83, 2866 - 2875.

Lykos, T., Varga, G.A., & Casper, D., 1997. Varying degradation rates of total nonstructural carbohydrates: effects on ruminal fermentation, blood metabolites, and milk production and composition in high producing holstein cows. J. Dairy Sci. 80(12), 3341 - 3355.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 2002. Evaluation of food: Digestibility. In: Animal Nutrition. (6th Ed.). Pearson Education Ltd., Edinburgh Gate, Harlow, Essex, UK. pp. 246 - 263.

Mertens, D.R., 1997. Creating a system for meeting the fibre requirements of dairy cows. J. Dairy Sci. 80, 1463 - 1481.

Mertens, D. R., Broderick, G.A. & Simons, R., 1994. Efficacy of carbohydrate sources for improving utilization of N in alfalfa silage. J. Dairy Sci. 77(Suppl. 1), 240 - 252.

Mohney, K., 2002. Synchronization of carbohydrate and protein metabolism by ruminal microbes in continuous culture. PhD thesis, North Carolina State University, Raleigh, North Carolina, USA. pp. 1 - 69.

Morrison, F.B., 1959. Feeds and feeding: A handbook for the student and stockman. (22nd Ed.). Eds. The Morrison Publishing Company. Clinton, Iowa, USA. 1165 pp.

Mould, F.L., 2003. Predicting feed quality - chemical analysis and *in vitro* evaluation. Field Crops Research 84(1), 31 - 44.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. (7th Rev. Ed.). National Academy. Press, Washington, D.C., USA. pp. 34 - 35.

Nocek, J. E. & Tamminga, S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74, 3598 - 3629.

Nombekela, S. W. & Murphy, M.R., 1995. Sucrose supplementation and feed intake of dairy cows in early lactation. J. Dairy Sci. 78, 880 - 885.

Paggi, R.A., Rodriguez, C. & Fay, J.P., 2005. Growth and pectate-lyase activity of the ruminal bacterium lachnospira multiparus in the presence of short-chain organic acids. Lett. Appl. Microbiol. 41, 434 - 439.

Pell, A.N. & Schofield, P., 1993. Computerized monitoring of gas production to measure forage digestion *in vitro*. J. Dairy Sci. 76, 1063 - 1073.

Pell, A.N., Pitt, R.E. Doane, P.H. & Shofield, P., 1998. The development, use and application of gas production techniques at Cornell University, USA. In: *In vitro* techniques for measuring nutrient supply to ruminants. Eds. Deaville, E.R., Owen, E., Adesogan, A.T., Rymer, C.,Huntington, J.A. & Lawrence, T.L.J. British Society of Animal Science, Edinburgh, UK. Publication 22, 45 - 54.

Russell, J.B., O'Connor, J.D., Fox, D.G., Van Soest, P.J. & Sniffen, C.J., 1992. A net carbohydrate and protein system for evaluating cattle diets: Ruminal fermentation. J. Anim. Sci. 70, 3551 – 3561.

Schwarz, F.J., Haffner, J. & Krichgessner, M., 1995. Supplementation of zero-grazed dairy cows with molassed sugar beet pulp, maize or cereal-rich concentrate. Anim. Feed Sci. Technol. 54, 237 - 248.

Stobel, H.J. & Russel, J.B., 1986. Effect of pH and energy spilling on bacterial proteien synthesis by carbohydrate-limited cultures of mixed rumen bacteria. J. Dairy Sci. 69, 2941 - 2947.

Stokes, S.R., 1997. Balancing carbohydrates for optimal rumen function and animal health. In: Proceedings of the West. Can. Dairy Sem. 9, 73 - 86.
Available at: http://www.wcds.afns.ualberta.ca/Proceedings/1997/ch06-97.htm
(Accessed 7 October 2008)

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B. & France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185 - 197.

Tilley, J.M.A. & Terry, R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18, 104 - 111.

University of Minnesota, 1996. Feeding the dairy herd (online)
Available at: http://www.extension.umn.edu/distribution/livestocksystems/components/DI0469-02
(Accessed 5 August 2008)

Van der Merwe, F.J. & Smith, W.A., 1991. Dierevoeding. Kosmo Publ., Stellenbosch, South Africa. pp. 89 - 141 (in Afrikaans).

Van Saun, R.J., 1993. Dairy cattle nutrition: feeding for two. In: Proceedings of the 1993 Northwest and Lower Columbia Dairy Short Course, January 21 - 22 & 25 - 26, Everett, Washington and Portland, Oregon, USA. pp. 172 - 180.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant. (2nd Ed.). Cornell University Press, Ithaca, New York, USA. pp. 467 - 476.

Van Soest, P.J. & Wine, R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Contamination of Plant Cell-wall Constituents. J. Assoc. Off. Anal. Chem. 50, 50 - 55.

Wilkins, R.J., 2000. Forages and their role in animal systems. In: Forage evaluation in ruminant nutrition. Eds. Givens, D.I., Owen, E., Axford, R.F.E., Omed, H.M. CAB International, Wallingford, UK. pp. 1 -14.

Zinn, R.A. & Ware, R.A., 2007. Forage quality: Digestive limitations and their relationships to performance of beef and dairy cattle. 22nd Annual Southwest Nutrition & Management Conference. February 22 - 23, University of California, Davis, Caifornia, USA.
Available at: http://ag.arizona.edu/ans/swnmc/2007/talks/Zinn_2007SWNMC.pdf
(Accessed 16 September 2008)

Chapter 3

THE EFFECT OF SUGAR, STARCH OR PECTIN ON RATE AND EXTENT OF GAS PRODUCTION IN DIFFERENT FORAGES

Abstract

The study evaluated the effect of sugar (molasses), starch (maize meal) or pectin (citrus pulp) on total gas production (b) and rate of gas production (c) of different forages. The forage substrates included wheat straw (WS), oat hay (OH), lucerne hay (LUC), ryegrass (RYE) and kikuyu grass (KIK). The three energy sources, as well as a control (no energy source) were incubated *in vitro* with each of the above mentioned forages. Rumen fluid was collected from two lactating Holstein cows receiving a diet consisting of oat hay, lucerne, wheat straw and a concentrate mix. Forages alone and/or together with either molasses, citrus pulp or maize meal were weighed into 100 ml glass vials and incubated for 72 hours. The weights of the energy sources were calculated on an energy equivalent basis. Blank vials, that contained no substrates, were included to correct for gas production from rumen fluid alone. The substrates were incubated in a mixture of 40 ml buffered medium, 2 ml of reducing solution and 10 ml rumen fluid. Gas pressure was recorded automatically every five minutes using a pressure transducer system and the method based on the Reading Pressure Technique. Gas pressure was converted to gas volume using a predetermined regression equation. In the first trial, the gas production included gas produced by the energy sources, while in the second trial, the energy source gas production was deducted from the total gas production to determine the effect of energy source on gas production of respective forage substrates *per se*. Data were fitted to two non-linear models. Significant forage x energy interactions were observed for the non-linear parameter b (gas production) in Model 1 and for b and L (lag phase) in Model 2 in both trials. In the first trial, the higher fermentability of the energy sources supplemented to forage substrates, increased b (Model 1 & 2) of the LUC and WS ($P < 0.05$). The gas production rate was affected in different ways for different forages, with the most noticeable effect when WS was supplemented with energy sources. All the energy sources increased c of WS irrespective of the model used. Energy sources had no effect on the L of LUC, OH or RYE but decreased the L of WS ($P = 0.004$) and KIK ($P = 0.022$). In the second trial, maize meal had no effect on b for any of the forages (Model 1 & 2), while molasses (Model 1 & 2) decreased b for all forage substrates, and citrus pulp (Model 1 & 2) decreased b of OH and RYE, to lower values than those of the control treatments. Gas production rate was not affected by molasses for any of the forage substrates, while citrus pulp (Model 1 & 2) increased c of OH and maize meal increased c of OH and KIK. Lag phase was only affected by energy sources in WS ($P = 0.001$) and KIK ($P = 0.009$), where all the energy sources had lower L values than the control treatment. It was concluded that forage fermentability is affected differently by different energy sources. These observations may have important implications for rumen health and milk production, and the data obtained can potentially be used as guidelines in feed formulations.

3.1. Introduction

Forages contribute significant major part of dairy cow diets. However, forages alone cannot provide all the energy requirements of high producing dairy cows (Schwarz *et al.*, 1995). Forage degradation by rumen micro-organisms is restricted by the chemical composition and physical quality of the forage (Mertens, 1997). Fibrous feeds are low in energy and a large consumption thereof results in rumen fill, thus limiting feed intake

(Allen & Oba, 2000). It is important to find ways to improve forage degradation and utilization. Improvements in forage degradation will increase energy intake and subsequently milk production and performance (Giraldo *et al.*, 2008).

Different sources of non-fibre carbohydrates (NFC) have different effects on animal performance. Supplementing forage based diets with energy sources containing sugar, starch or pectin results in variation of performance measurements such as milk yield, milk composition, dry matter intake (DMI) and feed efficiency (Larson, 2003). Aldrich *et al*. (1993) reported that feeding high rumen-available NFC, increased milk protein percentage slightly (3.01% vs. 3.07%), but significantly. Leiva *et al*. (2000) showed that substituting diets containing 20.5% citrus pulp for diets containing 19.5% corn meal, yielded more milk. Solomon *et al*. (2000) however, reported that substituting starch (maize) diets with pectin (citrus pulp) diets had no effect on milk yield, but increased linoleic acid concentration in milk fat. When formulating diets for dairy cows it is important to understand the digestion and metabolizable nutrient yield of the various NFC sources (Larson, 2003).

The objective of this study was to determine the impact of three energy sources, *viz.* maize meal (representative of starch), molasses (representative of sugar) and citrus pulp (representative of pectin), on fermentation kinetics of different forage substrates as determined by total gas production (b) and the rate of gas production (c). Forages commonly used in dairy cow diets in South Africa (wheat straw, oat hay, lucerne hay, kikuyu and ryegrass) were used as substrates.

3.2. Materials and methods

3.2.1. Study area

The study to evaluate the effect of supplementing forage based diets with sugar, starch and pectin on rumen kinetic parameters was conducted at Stellenbosch University, Stellenbosch, South Africa (33° 55′ 12″ S, 18° 51′ 36″ E).

3.2.2. Simulated diets

3.2.2.1. Basal forages

Five forages (Table 3.1) were used to prepare rations to simulate diets for lactating dairy cows.

Table 3.1 Forages used in simulation diets for lactating dairy cows.

Forage Type	Abbreviation
Wheat straw (*Triticum aestivum*)	WS
Oat hay (*Avena sativa)*	OH
Lucerne hay (*Medicago sativa*)	LUC
Ryegrass (*Lolium multiflorum*)	RYE
Kikuyu grass (*Pennisetum clandestinum*)	KIK

Rye and kikuyu grasses were harvested after four weeks of re-growth. All the forages were oven dried at 60ºC for 72 hours. Wheat straw, oat hay and lucerne hay were ground (Cyclotec 1093 mill) through a 2 mm screen. Rye and kikuyu grasses were obtained from the Outeniqua experimental farm (33° 57′ 0″ S, 22° 25′ 0″ E), situated just outside George, South Africa (33° 58′ 0″ S, 22° 27′ 0″ E). The rye and kikuyu grasses were already ground through a 1 mm screen when received.

3.2.2.2. Energy supplements

Three energy sources (Table 3.2) were selected as supplements to prepare rations that would simulate lactating cow diets.

Table 3.2 Energy sources used in simulating lactating dairy cow diets.

Energy type	Source	Abbreviation
Starch	Yellow maize meal (*Zea mays)*	Mm
Sugar	Molasses syrup	Mol
Pectin	Citrus pulp	Cp

These energy feedstuffs were sourced in the following forms: molasses as a syrup by-product from the processing of sugar cane (*Officinarum saccharum*); citrus pulp as a finely granulated residue by-product from the peel, pulp and seeds of oranges and grapefruit and yellow maize grain. The citrus pulp and maize was milled (Cyclotec 1093 mill) through a 1 mm screen.

3.2.2.3. Defining the diets

A total of 23 simulated diets (forages and forage-concentrate mixes) were prepared:

- 5 diets contained forage substrates only (Table 3.1).
- 3 diets contained energy sources only (Table 3.2).
- 15 diets contained a mixture of forages (Table 3.1) and energy sources (Table 3.2).

The final substrate compositions are indicated in Table 3.4.

3.2.3. Chemical analyses of forages and energy sources

Samples (1 g) of each forage type and energy sources (1 g) were accurately weighed and placed in a 100ºC conventional oven for 24 hours to determine dry matter (DM) content (AOAC, 1995; Method 930.15). Organic matter was determined by weighing 1 g of each feedstuff into crucibles and ashing the content at 500ºC in a muffle furnace for 6 hours (AOAC, 1995; Method 942.05).

The NDF content was determined by measuring 0.5 g of each feedstuff into F57 ANKOM fibre analysis bags. The bags were heat sealed and NDF determined using the method of Van Soest *et al*. (1991). Sodium sulfite (Na_2SO_3) was added to the NDF solution during digestion and heat-stable amylase was added during rinsing. Ether extract was determined using the AOAC method (AOAC, 1995; Method 920.39). About 2 g of ground sample was weighed into a thimble and samples were extracted with diethyl ether ($C_4H_{10}O$).

Acid detergent lignin (ADL) was determined by measuring 0.5 g of each substrate sample into separate F57 ANKOM fibre analysis bags. The bags were heat sealed and acid detergent fibre (ADF) determined, using the method of Van Soest *et al*. (1991). The ADF residue was then soaked in 72% sulphuric acid for three hours to dissolve the cellulose. Acid detergent lignin was determined using the ANKOM method (ANKOM, 2005).

Total nitrogen content was determined with a Leco Nitrogen Gas Analyzer custom designed and built by LECO Africa (Pty) Ltd (Kempton Park). About 0.1 g of sample was accurately weighed into a small square of aluminum foil. The samples were then ignited inside the Leco furnace at about 900°C according to the Dumas procedure (AOAC, 1990; Method 968.06). Crude protein (CP) was calculated by multiplying the nitrogen content with 6.25 (AOAC, 1995; Method 990.03). The results obtained from the chemical analysis are shown in Table 3.3.

3.2.4. Preparation of samples for gas production

The gas production method used was based on the Reading Pressure Technique (Mauricio *et al.,* 1999). Samples of the respective dietary substrates (Tables 3.4 and 3.5) were weighed into glass vials with known volume. The vials had a nominal volume of 100 ml, but the exact volume of each vial was previously accurately determined. The amount of the energy sources in Table 3.5 were calculated to provide the same amount of metabolizable energy (ME) than 0.125 g maize DM. For calculation purposes, ME values were assumed to be 13.9 MJ/kg DM for maize, 12.2 MJ/kg DM for citrus pulp and 12.3 MJ/kg for molasses (NRC, 2001). Blank vials were prepared exactly as the others, except that they did not contain any substrates, in order to correct for gas production from rumen fluid alone.

Table 3.3 Chemical composition (g/kg DM ± SD) of forages and energy sources used in the trial. All values are on a DM basis.

Feedstuff	DM	OM	NDF	CP	EE	ADL
WS	915.0 ± 0.5	902.2 ± 1.6	817.9 ± 7.1	72.3 ± 1.0	3.6 ± 0.5	102.2 ± 3.0
OH	850.4 ± 2.3	940.3 ± 17.3	781.9 ± 11.0	91.0 ± 1.2	9.7 ± 0.8	82.5 ± 0.3
LUC	898.9 ± 2.0	906.2 ± 1.8	515.3 ± 1.0	237.0 ± 4.6	8.1 ± 0.6	92.7 ± 6.7
RYE	93.8 ± 1.0	84.5 ± 0.5	489.2 ± 3.0	250.9 ± 2.7	43.1 ± 0.1	44.8 ± 18.1
KIK	124.2 ± 10.4	112.2 ± 0.1	651.2 ± 2.0	253.4 ± 4.1	28.3 ± 1.1	48.0 ± 1.7
mm	855.9 ± 0.9	984.4 ± 1.3	174.3 ± 55.0	107.1 ± 0.2	37.7 ± 0.4	7.0 ± 0.6
cp	879.9 ± 0.9	928.5 ± 0.7	261.4 ± 14.0	78.6 ± 1.8	16.4 ± 0.4	25.3 ± 6.4
mol	698.0 ± 1.9	892.7 ± 0.6	ND	ND	ND	ND

DM = dry matter; OM = organic matter; NDF = neutral detergent fibre; CP = crude protein; EE = ether extract; ADL = acid detergent lignin; ND = not determined; WS = wheat straw; OH = oat hay; LUC = lucerne; RYE = ryegrass; KIK = kikuyu grass; mm = maize meal; cp = citrus pulp; mol = molasses.

Table 3.4 Substrate samples containing either forage or energy supplements.

Forage type	Energy source	Sample size (g DM)
Wheat straw	-	0.2500
Oat hay	-	0.2500
Lucerne hay	-	0.2500
Ryegrass	-	0.2500
Kikuyu grass	-	0.2500
-	Maize meal	0.1250
-	Citrus pulp	0.1425
-	Molasses	0.1412

Table 3.5 Composite dietary samples containing forage and energy sources.

Forage type	Energy source	Forage (g DM)	Energy (g DM)
Wheat straw	Maize meal	0.1250	0.1250
Wheat straw	Citrus pulp	0.1250	0.1425
Wheat straw	Molasses	0.1250	0.1412
Oat hay	Maize meal	0.1250	0.1250
Oat hay	Citrus pulp	0.1250	0.1425
Oat hay	Molasses	0.1250	0.1412
Lucerne hay	Maize meal	0.1250	0.1250
Lucerne hay	Citrus pulp	0.1250	0.1425
Lucerne hay	Molasses	0.1250	0.1412
Ryegrass	Maize meal	0.1250	0.1250
Ryegrass	Citrus pulp	0.1250	0.1425
Ryegrass	Molasses	0.1250	0.1412
Kikuyu grass	Maize meal	0.1250	0.1250
Kikuyu grass	Citrus pulp	0.1250	0.1425
Kikuyu grass	Molasses	0.1250	0.1412

3.2.5. Preparation of the *in vitro* medium and reducing solution

The incubation medium was prepared as described by Van Soest & Robertson (1985). The medium consisted of micro minerals, macro-mineral solution, buffer solution, tryptose, rezasurin and distilled water. The medium was kept in a water bath at 39.5ºC. The pH of the medium was about 7.8. Reducing solution was prepared as described by Van Soest & Robertson (1985) and consisted of cysteine hydrochloride ($C_3H_7NO_2 \cdot HCL$), potassium hydroxide (KOH) pellets, sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) and distilled water.

3.2.6. Collection and preparation of rumen fluid

Rumen fluid was collected from two ruminally cannulated lactating Holstein cows. The cows were confined and received 25 kg per day (air dry basis) of a diet consisting of oat hay, lucerne hay, wheat straw and a concentrate mix. The total diet contained 112.79 g/kg CP, 559.48 g/kg NDF and 59.50 g/kg ash, with a calculated ME content of 10.8 MJ/kg. The diet was offered in two equal amounts, *viz.* 12.5 kg in the morning (06:30) and 12.5 kg in the afternoon (16:30). Rumen fluid was squeezed through two layers of cheese cloth into pre-warmed thermos flasks and a handful of solid material was added. The rumen fluid was then blended in a pre-warmed blender at a low speed for 10 seconds. The rumen fluid was then filtered through

four layers of cheese cloth into pre-warmed beakers while flushing with carbon dioxide (CO_2). The temperature of the rumen fluid averaged 38ºC and the pH averaged 5.8.

3.2.7. *In vitro* incubation

The glass vials were flushed with CO_2 while adding 40 ml of the medium and 2 ml of the reducing solution to each vial. A magnetic stirrer (0.2 ml) was also placed into each vial. The vials were then lightly closed with rubber stoppers and placed in the incubator at 39ºC until the medium was reduced (i.e. clear). Vials were re-opened and 10 ml of rumen fluid was added while flushing with CO_2. The vials were then closed tightly with rubber stoppers, crimp sealed and connected via needles to a pressure logging system. The vials were placed on magnetic stirrer plates in an incubator at 39ºC and were constantly stirred at a low speed. The material was incubated for 72 hours and gas pressure was recorded automatically every five minutes using a pressure transducer system that was custom designed and built by Eagle Technology (Pty) Ltd (Cape Town) based on the Reading Pressure Technique (RPT) (Mauricio *et al.,* 1999). Gas pressure was released on regular intervals to prevent pressure build-up in the vials.

3.2.8. Converting gas pressure to gas volume

Gas pressure data were converted to gas volume using the following linear regression equation developed by Goosen (2004) for Department of Animal Sciences' *in vitro* lab:

$$Y = \frac{[1000\ ((0.0977\ X)C)]}{OM}$$

Where: Y = Gas volume (ml/g OM)
X = Gas pressure (psi)
C = Vial head space (ml)
OM = Organic Matter (mg)

3.2.9. Estimating kinetic coefficients

Kinetic coefficients for gas production were derived from the gas volume data using the solver option in Excel and the non-linear models 1 and 2 (with and without a lag phase; respectively). The models are based on the modified version described by Ørskov and McDonald (1979).

Model 1: $$Y = b\left(1 - e^{-ct}\right)$$

Model 2: $$Y = b\left(1 - e^{-c(t-L)}\right)$$

Where: *Y* = gas volume at time t
b = total gas production
c = rate of gas production
t = incubation time
L = lag time

3.3. Statistical analysis

The first derivatives b and c (Model 1) and b, c and L (Model 2) were subjected to statistical analysis. The experiment was a two way cross classification and data was subjected to a factorial ANOVA with the factors forage and energy using Statistica 8.1 (2008). This was done for all the non-linear parameters. Main effects were interpreted in the cases where no interaction was observed. Significant forage x energy source interactions were observed for the non-linear parameter b in Model 1 and for b and L in Model 2. Therefore, a one-way ANOVA was done on each of the forages to determine the effect of energy sources. Differences between means were determined with a Tukey test and significance was declared at $P < 0.05$.

3.4. Results and discussion

3.4.1. Gas production, including that from the energy sources

Results of total gas (b) and rate (c) of gas production are presented in Table 3.6. Pasture grasses had higher gas volumes than mature forages before substitution with energy sources. Gas volume is generally a good indicator of digestibility, fermentability and microbial protein production (Sommart *et al.,* 2000). Substitution with energy sources tended to raise gas production (Table 3.6). When maize meal replaced 50% of the forage substrate, total 72 hour gas production was increased in the case of lucerne and wheat straw, irrespective of the model used. In wheat straw, total gas production was also increased with citrus pulp supplementation (Models 1 & 2) and with molasses (Model 2). For the other forages (oat hay, ryegrass and kikuyu), energy supplements did not have an effect on total gas production values. The total (72 hour) gas production values represent the sum of forage and energy source fermentations, except for the control

treatment where the substrates were forages only. The higher gas production values observed with energy supplementation (especially for wheat straw), was due to the energy sources being more readily fermentable and easily available to rumen micro-organisms. Energy sources are also low in NDF and ADL (Table 3.3), which would increase gas production as there exists a negative correlation between gas production and plant cell wall content (Sallam, 2005).

The rate of gas production (c) of forage substrates alone ranged between 0.03 and 0.09 ml/h, with lucerne and ryegrass having the highest rates. When the respective energy sources replaced 50% of the forage substrates, there were variable positive responses in terms of c of different forage substrates. In general, energy sources tended to raise the rate of gas production, probably due to the higher nutrient content and easier accessibility of chemical constitutes to rumen microbial enzymes as compared to the forage substrates alone (Arigbede *et al.,* 2006). In lucerne hay, molasses increased c compared to the control treatment (Model 1 & 2). Maize meal had no effect on c when compared to the control treatment. The latter agrees with Mertens & Loften (1980) where starch increased the lag time but had no effect on digestion rate of lucerne. This would suggest that the addition of energy sources to forage substrates do not decrease fibre digestibility by lowering the rate of fermentation. In oat hay, citrus pulp had the biggest effect on c (both models) while, with Model 1, molasses also increased c. Maize meal (both models) tended to increase c, but not significantly compared to the control treatment. The most noticeable effect of forage substitution with energy sources was observed for wheat straw. In this case, all the energy sources increased the rate of gas production, irrespective of the model used. While the other forages have moderate fermentation potentials (as can be seen from the b and c values), the fermentability of wheat straw is low, resulting in a higher response when energy was supplemented. In ryegrass, maize meal had no effect on c (Model 1 & 2) while citrus pulp (Model 1) and molasses (both models) improved c compared to the control treatment. Citrus pulp increased gas production rate in kikuyu ($P < 0.05$) when Model 1 was used, while maize meal and molasses only tended to increase c. Supplementation with citrus pulp tended to improve gas production rates of forages more than maize meal. Citrus pulp is high in degradable NDF, resulting in less negative effects on cellulolytic bacteria and the ruminal environment than starch supplementations (Bampidis & Robinson, 2006). Unlike sugars and starches, pectin substances do not lower the rumen pH (Mohney, 2002). Pectin supplementation would thus sustain an optimal ruminal environment for cellulolytic bacteria functions, explaining the better gas production rates resulting from forages supplemented with citrus pulp. Leiva *et al*. (2000) also reported that citrus pulp ferment at a faster rate than corn hominy. Energy source had no significant effect on gas production rate of kikuyu in Model 2. It thus seems that energy sources *per se* tended to improve forage gas production rate. Energy sources are high in nutrients that are easily available and rapidly fermentable by rumen micro-organisms. Hiltner & Dehority (1983) found that forage digestion improved with energy sources supplementation due to an increase in the number of rumen micro-organisms available to help with fermentation.

When the respective energy sources replaced 50% of the forage substrates, a significant forage x energy source interaction was observed for the lag phase. Different lag times of forage substrates (control treatments) may be the result of differences in plant tissue composition between forages that require different degrees of physical or chemical changes before rumen micro-organisms can start with fibre digestion (Mertens & Loften, 1980). Lucerne fermented alone had almost no lag phase, but supplementation of maize

meal tended (P = 0.069) to increase the lag phase. Adesogan *et al*. (2005) found that maize and citrus pulp incubated individually, had longer lag phases when compared to hays (P < 0.001), agreeing with results obtained in this study that the energy sources tended to lengthen the lag phase of lucerne hay. The longer lag phase for the maize and citrus pulp treatments might be associated with a high proportion of cellulolytic micro-organisms in the rumen fluid. The diet of the cannulated cows consisted predominantly of oat hay, lucerne hay and wheat straw and might have resulted in insufficient numbers of pectin-fermenting and amylolytic bacteria in the collected rumen fluid to instantly colonize the citrus pulp and maize, respectively (Adesogan *et al*., 2005). The results also agree with the work of Mertens & Loften (1980) where starch increased the lag time but had no effect on digestion rate of lucerne. Possible reasons include a delay in fermentation due to microbial colonization (Chesson & Forsberg, 1988). In contrast, Haddad & Grant (2000) found that a reduction in NFC content substituted to lucerne based diets *in vitro*, increased the lag time of lucerne.

Table 3.6 Effects of maize meal, citrus pulp and molasses as energy sources on fermentation kinetics of different forage substrates, as measured by *in vitro* gas production. Gas production of both energy sources and forage substrates are included.

	Treatment					
Item	**Maize meal**	**Citrus pulp**	**Molasses**	**Control**	**SEm**	**P**
Lucerne hay						
Model 1:						
b	398.1 ^{b}	350.0 ^{ab}	334.2 ^{ab}	270.1 ^{a}	24.98	0.025
c	0.09 ^{a}	0.11 ^{ab}	0.14 ^{b}	0.09 ^{a}	0.01	0.005
Model 2:						
b	396.5 ^{b}	349.0 ^{ab}	334.0 ^{ab}	270.0 ^{a}	25.13	0.028
c	0.10 ^{a}	0.11 ^{ab}	0.15 ^{b}	0.09 ^{a}	0.01	0.013
L	0.45	0.33	0.09	0.05	0.11	0.069
Oat hay						
Model 1:						
b	398.2	365.4	315.0	296.6	37.2	0.250
c	0.08 ^{ab}	0.11 ^{b}	0.08 ^{b}	0.04 ^{a}	0.01	0.002
Model 2:						
b	397.1	365.4	315.0	295.1	37.45	0.253
c	0.08 ^{ab}	0.11 ^{b}	0.08 ^{ab}	0.05 ^{a}	0.01	0.004
L	0.32	0	0	0.36	0.13	0.125

Table 3.6 (continue) Effects of maize meal, citrus pulp and molasses as energy sources on fermentation kinetics of different forage substrates, as measured by *in vitro* gas production. Gas production of both energy sources and forage substrates are included.

Wheat straw						
Model 1:						
b	370.6 [b]	359.1 [b]	306.2 [ab]	246.0 [a]	18.76	0.002
c	0.08 [b]	0.10 [b]	0.09 [b]	0.03 [a]	0.01	0.001
Model 2:						
b	369.6 [b]	359.1 [b]	306.2 [b]	228.9 [a]	17.96	0.001
c	0.08 [b]	0.10 [b]	0.09 [b]	0.03 [a]	0.01	0.001
L	0.32 [b]	0.00 [b]	0.00 [b]	2.25 [a]	0.40	0.004
Ryegrass						
Model 1:						
b	449.2	411.3	377.7	372.7	34.54	0.406
c	0.08 [a]	0.12 [b]	0.12 [b]	0.08 [a]	0.01	0.001
Model 2:						
b	447.1	410.6	377.6	371.1	34.75	0.422
c	0.08 [a]	0.12 [cb]	0.12 [b]	0.09 [ac]	0.01	0.006
L	0.51	0.24	0.03	0.54	0.18	0.213
Kikuyu grass						
Model 1:						
b	423.6	426.9	390.2	323.0	26.34	0.055
c	0.09 [ab]	0.11 [b]	0.10 [ab]	0.05 [a]	0.01	0.025
Model 2:						
b	422.4	426.6	390.2	377.6	24.03	0.425
c	0.10	0.11	0.10	0.06	0.01	0.059
L	0.26 [ab]	0.11 [b]	0.00 [b]	1.63 [a]	0.35	0.022

b = Total gas production (ml/g OM); c = gas production rate (ml/h); L = Lag time (h);
Model 1: $Y = b + (1 – e^{-ct})$; Model 2: $Y = b + (1 – e^{-c(t-L)})$; Superscripts are comparing across rows.

The lag phase for oat hay was about 22 minutes and was not significantly affected by energy source. The greatest effect of forage substitution with energy sources was observed for wheat straw, where all the energy sources shortened the lag phase, compared to the control treatment where wheat straw had a lag phase of more than 2 hours. In ryegrass, treatment had no significant effect on lag phase, but molasses tended to decrease the lag phase. In kikuyu, the lag phase was significantly shortened when supplemented with citrus pulp and molasses ($P < 0.05$), while maize meal only tended to shorten the lag. Hiltner & Dehority (1983) also found a decrease in lag times of fibre digestion when soluble carbohydrates were added to *in vitro* incubations. They concluded that the decrease in lag phase was due to an increase in bacteria numbers helping with digestion, as they found similar results when they increased the inoculum.

The parameters presented in Table 3.6 (Model 2) were used to construct Figures 3.1 - 3.6. The data presented in the figures represent total gas production including that of the energy sources.

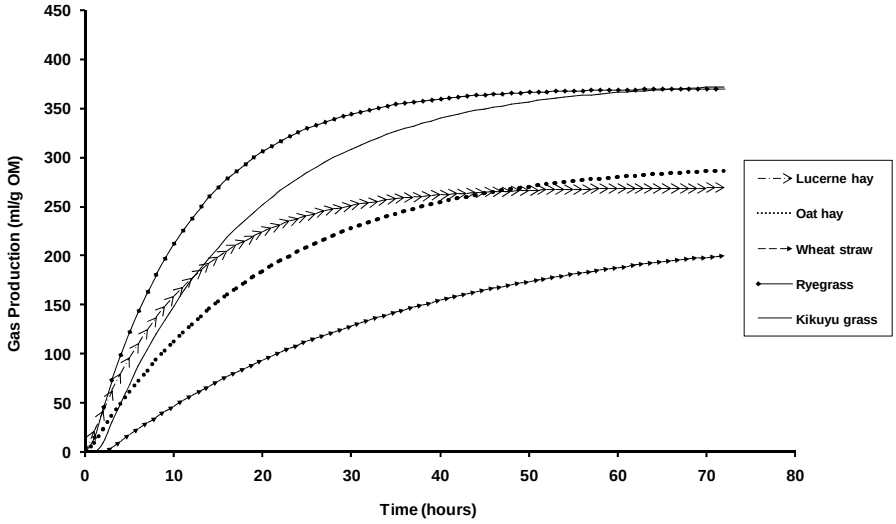

Figure 3.1 Gas production of forage substrates alone.

Figure 3.1 show differences in fermentation characteristics of the different forages. Variations in NDF, ADL and CP contents of these forages (Table 3.3), as well as different arrangements of their cell-wall polysaccharides (Cheng *et al.,* 1984), are most likely responsible for different fermentation characteristics observed between forage substrates. The fermentation patterns of the forages are functions of the forage type, as well as the physiological stage at which they were harvested, both of which affect their chemical composition. The grasses (ryegrass and kikuyu) were immature (28 days of re-growth). Lucerne and oat hay were harvested at the 10% flowering stage, while wheat straw was a mature forage. As forages mature, their NDF and ADL contents increase (McDonald *et al.*, 2002). Wheat straw had the highest NDF and ADL contents (Table 3.3). Although lucerne hay had a relatively low NDF content, its ADL content was quite high, compared to kikuyu which had a fairly high NDF content, but a low ADL content, resulting in the early cut grasses to manifest a much higher fermentability profile than the other forages. Figure 3.1 clearly indicates that wheat straw has a much lower fermentability, both in terms of total gas production and rate of gas production, than the other forages. Wheat straw also has a much longer lag phase compared to other forages. The lower fermentability and longer lag phase of wheat straw, which is a mature forage, can be explained by the tissue content of wheat straw that is high in NDF and ADL (which is negatively correlated with gas production), subsequently requiring more physical and chemical alterations before bacteria in the rumen can start digestion. The high rate and extent of gas production from both ryegrass and kikuyu can also be observed (Figure 3.1). Apart from readily fermentable fibre, these pasture grasses are also high in nitrogen (Table 3.3) and total carbohydrates, which are both needed for optimal growth of rumen micro-organisms and thus fermentation (NRC, 2001).

The effect of energy sources on the fermentation profiles of the different forages can be observed in Figures 3.2 - 3.6.

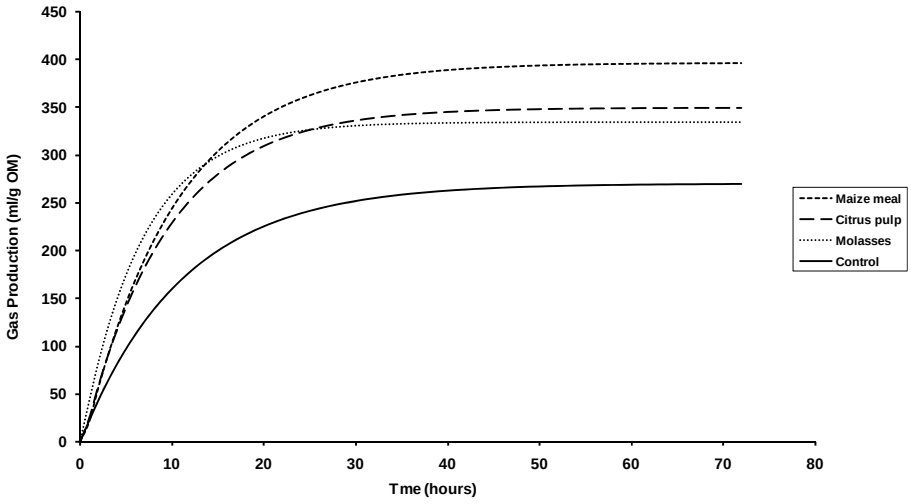

Figure 3.2 Gas production of lucerne hay when supplemented with different energy sources.

Substituting 50% of the forages with an energy source improved gas production (Figures 3.2 - 3.6), probably by boosting microbial growth. Maize meal and citrus pulp had the biggest effect throughout. Molasses is a fast fermenting simple sugar (Hall, 2002), which is rapidly utilized, explaining the fast increase in gas production followed by a plateau soon afterwards. Maize meal and citrus pulp, other than molasses, are more complex energy sources, which ferment slower and are available over a longer period of time. As mentioned before, wheat straw was a mature forage compared to ryegrass and kikuyu which were harvested after only 28 days of re-growth, while lucerne and oat hay were harvested at 10% flowering stage. Ryegrass, lucerne and kikuyu are high in nitrogen (Table 3.3). Sufficient nitrogen ensures nitrogen-energy coupling to occur at a greater extent, thereby ensuring more efficient microbial fermentation and cellulose degradation by rumen bacteria (NRC, 2001). As forages mature, the NDF and ADF contents increase and CP (supplying nitrogen) decreases, making less nitrogen readily available to rumen micro-organisms for fermentation (Ghadaki *et al.*, 1975). This may explain why maize meal and citrus pulp had higher impacts than molasses on gas production of the mature forages. Citrus pulp and maize meal release energy at a slower rate, which match the slow release of nitrogen in mature forages. Also, even though small these energy sources contributes rumen degradable protein (RDP), which with substrates like wheat straw (that has very low CP content) could be quite substantial. It should be kept in mind though, that the gas production profiles in these figures reflect the combination of forages and energy sources.

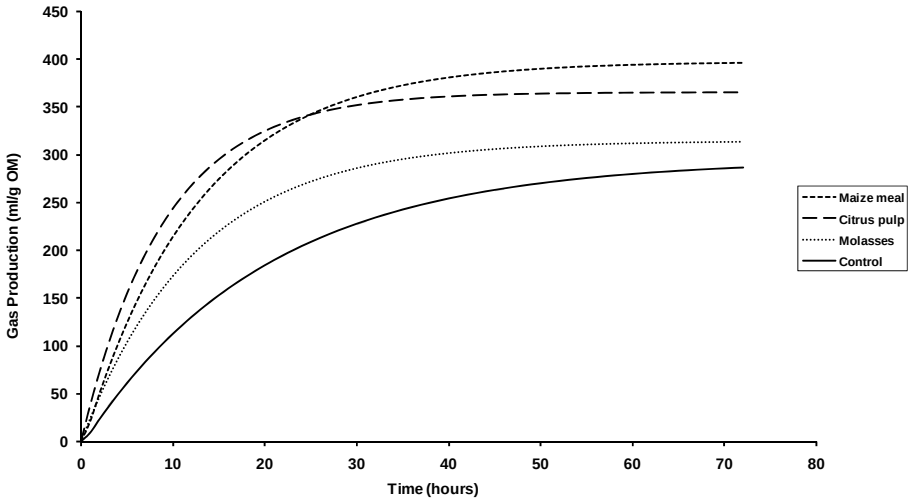

Figure 3.3 Gas production of oat hay when supplemented with different energy sources.

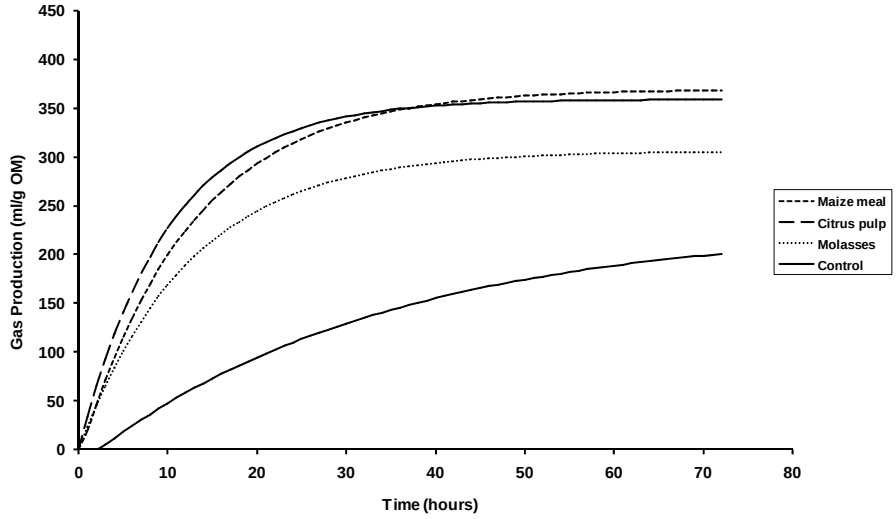

Figure 3.4 Gas production of wheat straw when supplemented with different energy sources.

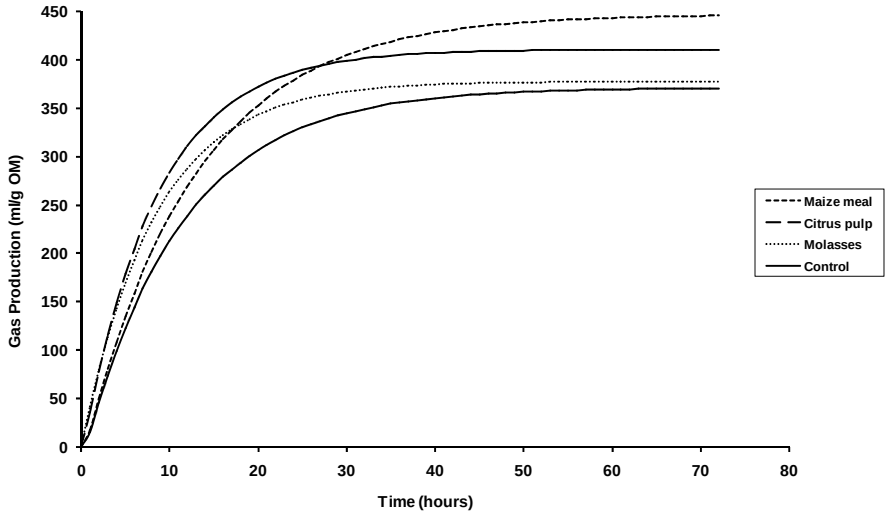

Figure 3.5 Gas production of ryegrass when supplemented with different energy sources.

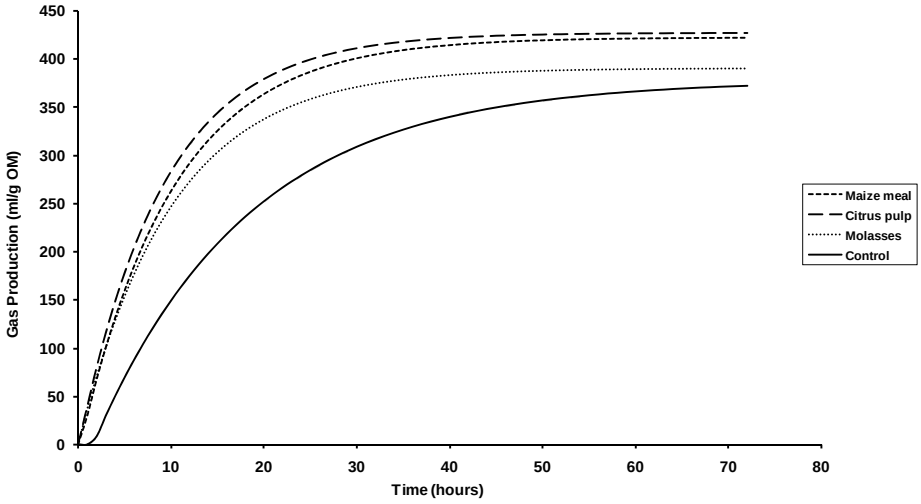

Figure 3.6 Gas production of kikuyu grass when supplemented with different energy sources.

3.4.2. Gas production parameters including that of forage and energy sources in cases where no interaction was observed

In the cases where *in vitro* gas production parameters showed no interactions, the main effects are discussed. Gas production values of the different forages (Models 1 & 2) differed ($P < 0.05$) from each other

independent of the energy source supplemented (Table 3.7). The NDF, ADL and CP content of the forages differed (Table 3.3). The differences in chemical and physical tissue structure probably influenced their fermentation kinetic patterns (Figure 3.1). In Model 1, ryegrass resulted in higher gas productions than oat hay, wheat straw and lucerne, but did not differ from kikuyu. In Model 2, the same trend was seen for gas production, with kikuyu and ryegrass having higher values than lucerne, oat hay and wheat straw. The reason for the higher gas production from ryegrass compared to lucerne, wheat straw and oat hay are due to the lower NDF and ADF contents of ryegrass resulting in higher gas production (Sallam, 2005). Ryegrass and kikuyu, as mentioned before, were harvested young compared to the other forages. These pasture grasses therefore had more rapidly fermentable sugar resulting in higher gas production values. Ryegrass and kikuyu are also high in CP (Table 3.3) which is essential for optimal rumen fermentation, as it supplies the rumen micro-organisms with nitrogen that is important for their growth. Gas production rates differed for among forages ($P < 0.05$), irrespective of the energy source. Ryegrass and lucerne had higher c values than oat hay and wheat straw.

Table 3.7 *In vitro* gas production parameters of forages, as affected by energy sources as main effects in cases where no interactions were observed. Gas production of both energy sources and forage substrates are included.

	Forage						
Item	**Lucerne hay**	**Oat hay**	**Wheat straw**	**Ryegrass**	**Kikuyu grass**	**SEm**	**P**
Model 1							
b	338.1 ^{ac}	343.8 ^{ac}	320.5 ^{a}	402.7 ^{b}	390.9 ^{bc}	14.57	<0.001
Model 2							
b	337.4 ^{a}	343.1 ^{a}	315.9 ^{a}	401.6 ^{b}	404.2 ^{b}	14.39	<0.001
c	0.11 ^{b}	0.08 ^{a}	0.08 ^{a}	0.10 ^{bc}	0.09 ^{ac}	0.01	<0.001

b = Total gas production (ml/g OM); c = gas production rate (ml/h);
Model 1: $Y = b + (1 – e^{-ct})$; Model 2: $Y = b + (1 – e^{-c(t-L)})$; Superscripts are compared across rows.

The total gas production values of forage substrates (Models 1 & 2) differed ($P < 0.05$) for each energy source supplemented (Table 3.8). Maize meal and citrus pulp supplementations resulted in higher forage gas production values than the control treatment (Model 1 & 2). Maize meal tended to have higher gas production than citrus pulp, supporting the theory of Sallam (2005) that feedstuffs higher in NDF and ADF content result in lower gas production. Gas production rate (c) of the forages were all higher for the energy source treatments than that of the control treatment ($P < 0.05$), indicating the faster fermentation potential of the energy sources. The highest gas production rates were achieved when forages were supplemented with citrus pulp and molasses, irrespective of the forage substrate used. Pectin substances, other than starches and sugars, produce no lactic acid (Van Soest, 1994). Pectin subsequently does not tend to lower the rumen pH as much as sugars and starches, thus sustaining optimal ruminal environments for cellulolytic bacteria. This could partly explain the better gas production rates of forages supplemented with citrus pulp.

Table 3.8 *In vitro* gas production parameters of forages (irrespective of forage substrate used) as affected by different energy sources as main effects in cases where no interactions were observed. Gas production of both energy sources and forage substrates are included.

	Energy source					
Item	**Maize meal**	**Citrus pulp**	**Molasses**	**Control**	**SEm**	**P**
Model 1						
b	407.9 [b]	382.5 [bc]	344.6 [ac]	301.7 [a]	13.03	<0.001
Model 2						
b	406.5 [b]	382.1 [bc]	344.6 [ac]	308.5 [a]	12.87	<0.001
c	0.09 [b]	0.11 [c]	0.11 [c]	0.06 [a]	0.00	<0.001

b = Total gas production (ml/g OM); c = gas production rate (ml/h);
Model 1: $Y = b + (1 – e^{-ct})$; Model 2: $Y = b + (1 – e^{-c(t-L)})$; Superscripts are compared across rows.

3.4.3. Gas production, excluding that from energy sources

Gas production values from the respective energy sources alone were obtained from separate fermentations where forage substrates were omitted. These values were subtracted from total gas production values where forages and energy sources were incubated together, to calculate the effect of energy sources on gas production of the respective forage sources *per se* (Table 3.9).

When the maize meal gas production was deducted from the total gas production, there was no difference between the control and the maize meal supplemented treatments in any of the forages (Models 1 & 2). When molasses gas production was deducted from the total gas production, however, total gas production from forages in the molasses supplemented treatments was lower than that of the control treatments in all forage substrates, irrespective of the model used. In oat hay, molasses lowered gas production more than in any of the other treatments, including the control treatment. In oat hay and ryegrass (both models), and in lucerne hay (Model 2), citrus pulp as energy source also decreased forage gas production compared to the control treatment. The lower gas production after energy sources gas production was deducted illustrate that energy sources were the main reason for the higher gas production presented earlier in treatments with forage and energy source combinations as substrates. Gas production is negatively correlated with NDF and ADL content (Sallam, 2005). Energy sources had lower NDF and trace amounts of lignin content when compared to forage substrates (Table 3.3), supporting the theory of higher gas production observed after energy inclusions in the simulated dairy cow diets. Energy sources *per se*, thus seemed to increase the rate of forage digestion, but maintained or decreased the digestibility of forages. Possible reasons for the lower digestibility, as determined by gas production, can be because micro-organisms first attack the easily fermentable energy sources before starting with fibre digestion.

Deducting energy source gas production resulted in variable positive responses in terms of rate of gas production from forage substrates. In oat hay, maize meal and citrus pulp treatments both improved c compared to the control treatment (Models 1 & 2). In kikuyu grass, maize meal had a profound effect and increased c when compared to the control treatment. For the other forages (lucerne, wheat straw, and ryegrass) deduction of the energy sources had no effect on fermentation rate.

Deducting energy source gas production values indicated that treatment with energy sources affected the lag phase differently for the different forages. Lucerne hay, oat hay and ryegrass had short lag phases which were not affected by energy source. Citrus pulp and maize meal, however, tended to increase the lag phase of lucerne hay. Similar results were obtained by Adesogan *et al.* (2005) who found that maize and citrus pulp resulted in a longer lag phase than hays ($P < 0.001$). The diet of the cows consisted predominantly of oat hay, lucerne hay and wheat straw. The diet of donor cows thus had high proportions of cellulolytic micro-organisms and less pectin-fermenting and amylolytic bacteria needed to colonize citrus pulp and maize meal respectively, explaining the longer lag phase of maize meal and citrus pulp (Adesogan *et al*., 2005). Kikuyu and wheat straw had long lag phases compared to the other forages. All the energy sources significantly shortened the lag phase of wheat straw and kikuyu.

Table 3.9 Effects of maize meal, citrus pulp and molasses as energy sources on fermentation kinetics of different forage substrates, as measured by *in vitro* gas production. Gas production resulting from energy sources was deducted from total gas production.

	Treatment					
Item	**Maize meal**	**Citrus pulp**	**Molasses**	**Control**	**SEm**	**P**
Lucerne hay						
Model 1:						
b	229.3 [ab]	197.4 [ab]	178.9 [b]	283.7 [a]	20.63	0.018
c	0.09	0.10	0.15	0.09	0.02	0.103
Model 2:						
b	219.2 [ab]	195.9 [b]	178.9 [b]	283.6 [a]	18.36	0.008
c	0.12	0.11	0.15	0.09	0.02	0.189
L	0.23	0.76	0.06	0.05	0.23	0.166
Oat hay						
Model 1:						
b	284.2 [ab]	236.7 [b]	176.1 [c]	328.3 [a]	13.15	0.001
c	0.08 [b]	0.10 [b]	0.04 [a]	0.04 [a]	0.01	0.001
Model 2:						
b	284.2 [ab]	236.7 [b]	176.1 [c]	327.3 [a]	13.17	0.001
c	0.08 [b]	0.10 [b]	0.04 [a]	0.04 [a]	0.01	0.001
L	0.00	0.00	0.00	0.17	0.07	0.240

Table 3.9(continue) Effects of maize meal, citrus pulp and molasses as energy sources on fermentation kinetics of different forage substrates, as measured by *in vitro* gas production. Gas production resulting from energy sources was deducted from total gas production.

Wheat straw						
Model 1:						
b	210.8 ^{ab}	190.3 ^{ab}	151.0 ^{b}	249.9 ^{a}	16.57	0.009
c	0.08	0.07	0.06	0.02	0.02	0.110
Model 2:						
b	210.8 ^{ab}	190.3 ^{ab}	151.0 ^{b}	231.2 ^{a}	15.59	0.020
c	0.08	0.07	0.06	0.03	0.02	0.168
L	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}	2.29 ^{a}	0.36	0.001
Ryegrass						
Model 1:						
b	284.5 ^{ab}	242.9 ^{b}	209.3 ^{b}	399.5 ^{a}	29.1	0.003
c	0.08	0.10	0.11	0.08	0.01	0.324
Model 2:						
b	283.5 ^{ab}	241.9 ^{b}	209.2 ^{b}	398.3 ^{a}	29.34	0.004
c	0.09	0.10	0.11	0.09	0.01	0.459
L	0.40	0.62	0.05	0.39	0.29	0.588
Kikuyu grass						
Model 1:						
b	250.0 ^{ab}	261.1 ^{ab}	219.3 ^{b}	334.9 ^{a}	23.69	0.028
c	0.12 ^{b}	0.10 ^{ab}	0.08 ^{ab}	0.05 ^{a}	0.01	0.017
Model 2:						
b	249.9 ^{ab}	261.0 ^{ab}	219.3 ^{b}	324.3 ^{a}	24.08	0.050
c	0.12 ^{b}	0.10 ^{ab}	0.08 ^{ab}	0.05 ^{a}	0.02	0.040
L	0.07 ^{b}	0.12 ^{b}	0.00 ^{b}	1.73 ^{a}	0.34	0.009

b = Total gas production (ml/g OM); c = gas production rate (ml/h); L = Lag time (h);
Model 1: $Y = b + (1 - e^{-ct})$; Model 2: $Y = b + (1 - e^{-c(t-L)})$; Superscripts are compared across rows.

Hiltner & Dehority (1983) similarly found that the addition of soluble carbohydrates to *in vitro* incubations, decreased the lag time of fibre digestion. They concluded that supplementation increased bacteria numbers thus decreasing the lag phase by supporting fibre digestion. They found similar results with increased amounts of inoculum. A possible reason for the long lag phase of kikuyu grass may be because it was heavily fertilized with nitrogen. Marais *et al*. (1988) showed that nitrite derived from high nitrate pastures resulted in reduced *in vitro* digestibility as it affected rumen microbial function. The long lag phase of wheat straw was probably due to the high NDF content which is slowly digested, and the high ADL content which is not digested by rumen micro-organisms. The latter could also have an effect on the structure of the fibre matrix, making it more difficult for rumen organisms to reach the more digestible fibre fractions. The short lag phases of lucerne and oat hay could partially be explained by the fact that the cannulated cows received diets containing these substrates, but also because their chemical composition allows higher digestibility compared to wheat straw. In general, it would appear that the supplementation of forages with various

energy sources had a negative effect on total gas production, but increased the rate of gas production in some instances.

Profiles of forage fermentation, after deduction of energy source values, are illustrated in Figures 3.7 – 3.12.

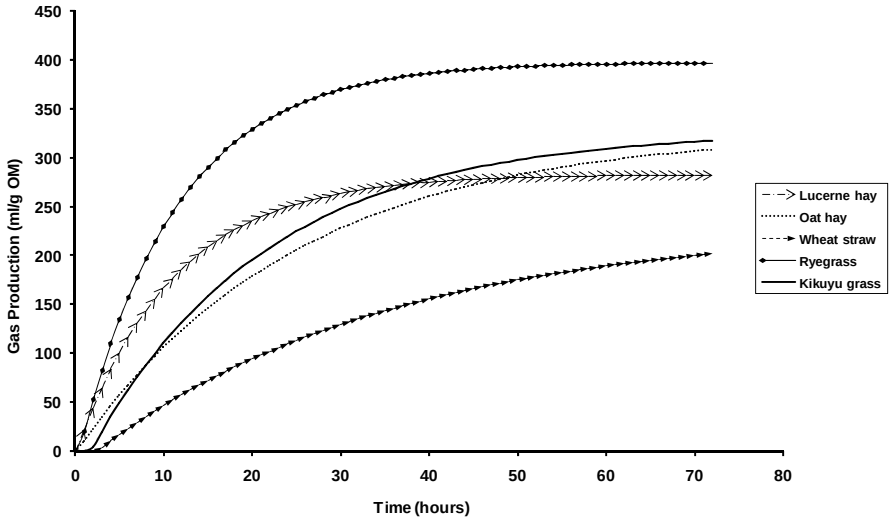

Figure 3.7 Gas production of forage substrates after gas production of energy sources has been deducted.

Variations in fermentation patterns of different forages are illustrated in Figure 3.7. All the forages differed in terms of gas production, lag phase and gas production rate. This is probably due to differences in their cell-wall polysaccharide arrangements (Cheng et al., 1984), as well as NDF, ADL and CP composition (Table 3.3). It is clear that wheat straw has a much lower fermentability, both in terms of total gas production and rate of gas production, than the other forages. As forages mature, their NDF and ADL contents increase (McDonald *et al.*, 2002). Wheat straw had the highest NDF and ADL contents (Table 3.3). The NDF and ADF contents of diets are negatively correlated with gas production (Sallam, 2005), which would explain the lower gas production of wheat straw in comparison with the other forages. Wheat straw is also very low in CP (Table 3.3). Crude protein provides rumen micro-organisms with nitrogen needed for optimal growth and fermentation. Ryegrass and kikuyu show a high rate and extent of gas production, maybe due to the lower NDF and ADL and higher CP contents as compared to wheat straw. Although lucerne hay had a relatively low NDF content, its ADL content was quite high, compared to kikuyu which had a higher NDF content, but a low ADL content, resulting in the early cut grasses to manifest a much higher fermentability profile than the other forages.

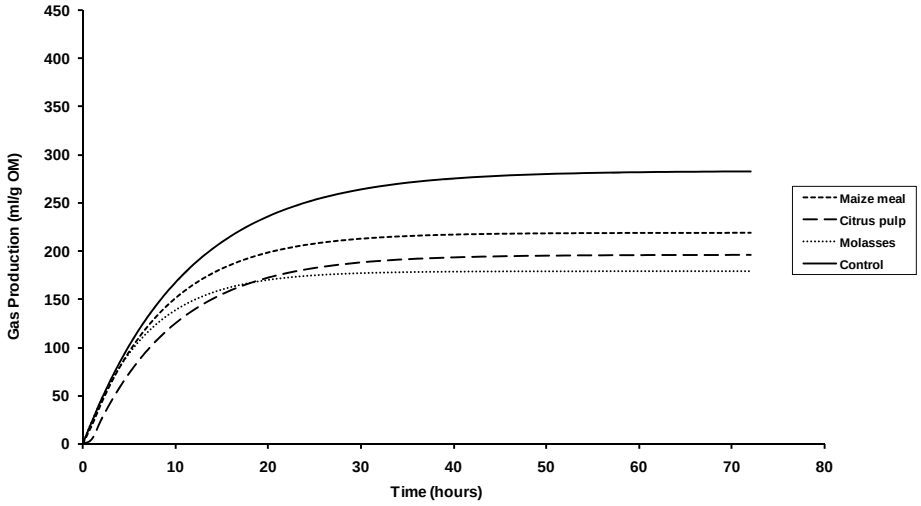

Figure 3.8 The net effect of energy supplements on gas production of lucerne hay.

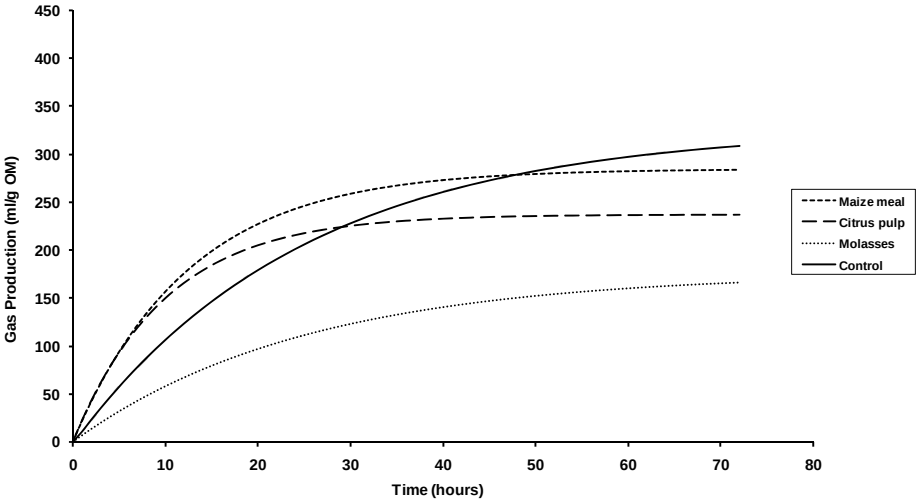

Figure 3.9 The net effect of energy supplements on gas production of oat hay.

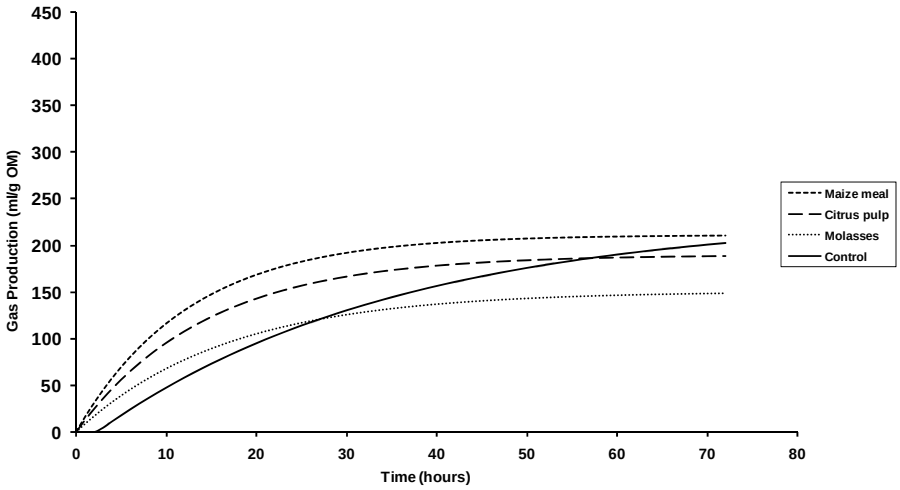

Figure 3.10 The net effect of energy supplements on gas production of wheat straw.

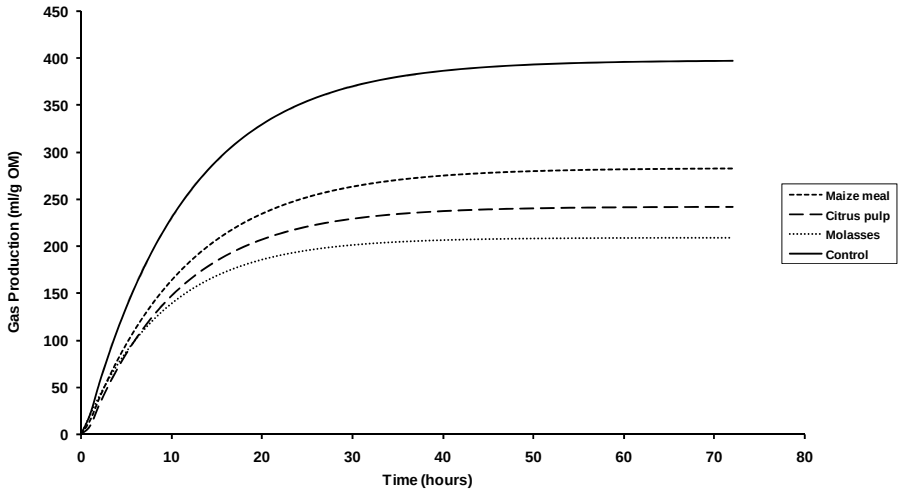

Figure 3.11 The net effect of energy supplements on gas production of ryegrass.

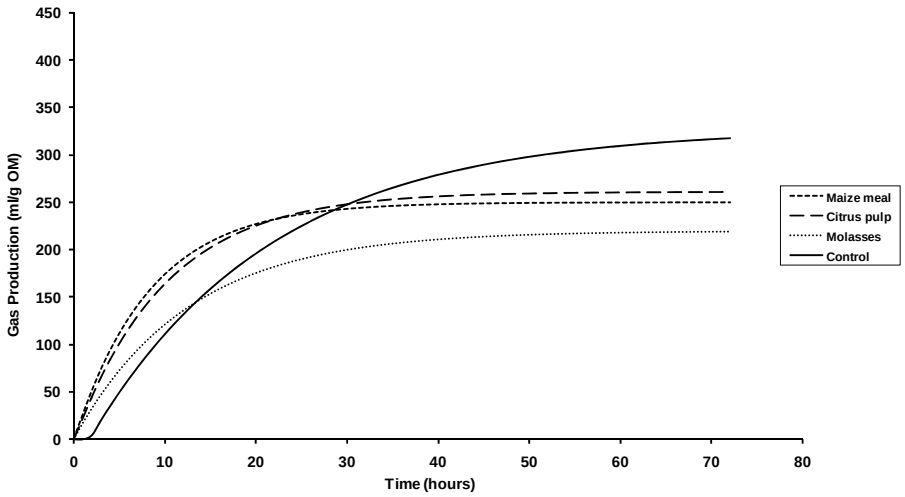

Figure 3.12 The net effect of energy supplements on gas production of kikuyu grass.

In many of these figures, the control clearly did not reach a plateau in the 72 hour incubation period. This enforces the findings that the energy sources increased the rate of gas production, especially during the earlier parts of the incubation.

3.4.4. Gas production parameters excluding that of energy sources in cases where no interaction was observed

The *in vitro* gas parameters that showed no interactions were interpreted in terms of main effects. Gas production values from the energy sources were deducted from the total gas production to derive forage gas production values (Table 3.10). It can be seen that the gas production values of the forage substrates differed ($P < 0.05$) from each other, independent of the deducted energy source (Models 1 & 2). Gas production resulting from wheat straw differed from ryegrass, oat hay and kikuyu, but not from lucerne (both models). The higher gas production of ryegrass and kikuyu, compared to wheat straw, results from the higher NDF and ADF contents of wheat straw which negatively affected its gas production (Sallam, 2005). Rate of gas production (c) also differed ($P < 0.05$) between forages, independent of energy source (Model 2). Oat hay and wheat straw had lower c-values than ryegrass and lucerne, but did not differ from kikuyu. The lower gas production rate of wheat straw and oat hay can be due to the maturity of these forages. These forages contain less readily fermentable substrates and more NDF and ADL compared to immature ryegrass and kikuyu cut at 28 days of re-growth.

Table 3.10 *In vitro* gas production parameters of forages, as affected by energy sources as main effects in cases where no interactions were observed. Gas production resulting from energy sources was deducted from total gas production.

	Forage						
Item	**Lucerne hay**	**Oat hay**	**Wheat straw**	**Ryegrass**	**Kikuyu grass**	**SEm**	**P**
Model 1							
b	222.3 ^{ab}	256.3 ^{bc}	200.5 ^{a}	284.0 ^{c}	266.3 ^{c}	10.68	<0.001
Model 2							
b	219.4 ^{ab}	256.1 ^{bc}	195.9 ^{a}	283.2 ^{c}	263.6 ^{c}	10.47	<0.001
c	0.12 ^{b}	0.07 ^{a}	0.06 ^{a}	0.10 ^{b}	0.09 ^{ab}	0.01	<0.001

b = Total gas production (ml/g OM); c = gas production rate (ml/h);
Model 1: $Y = b + (1 – e^{-ct})$; Model 2: $Y = b + (1 – e^{-c(t-L)})$; Superscripts are compared across rows.

The effect of energy source treatment on forage fermentation is presented in Table 3.11. Energy sources differed in terms of total gas produced by forages as a group. It appeared that deduction of gas production resulting from energy supplementation lowered total gas production of forages when either of the two models was used. Molasses resulted in the lowest total gas production from forages. With Model 2, all the energy sources increased the rate of fermentation of forage substrates, but no differences occurred between energy sources. The increase in fermentation rates might suggest that energy supplementation does not necessarily result in lower fibre digestion as observed by Mertens & Loften (1980), by reducing their fermentation rates (Miller & Muntifering, 1985). The increase in rate of gas production of forages after energy sources substitution may be due to their higher nutrient content and easier accessibility of the energy sources chemical constitutes to rumen microbial enzymes (Arigbede *et al.,* 2006).

Table 3.11 *In vitro* gas production parameters of forages (irrespective of forage substrate used) as affected by different energy sources as main effects in cases where no interactions were observed. Gas production resulting from energy sources was deducted from total gas production.

	Energy source					
Item	**Maize meal**	**Citrus pulp**	**Molasses**	**Control**	**SEm**	**P**
Model 1						
b	251.8 ^{b}	225.7 ^{b}	186.9 ^{c}	319.3 ^{a}	9.55	<0.001
Model 2						
b	249.5 ^{b}	225.1 ^{b}	186.9 ^{c}	313.0 ^{a}	9.37	<0.001
c	0.10 ^{b}	0.10 ^{b}	0.09 ^{b}	0.06 ^{a}	0.01	<0.001

b = Total gas production (ml/g OM); c = gas production rate (ml/h);
Model 1: $Y = b + (1 – e^{-ct})$; Model 2: $Y = b + (1 – e^{-c(t-L)})$; Superscripts are compared across rows.

3.5. Conclusion

Forage diets alone do not provide in the high energy requirements of lactating cows. Lactating cows produce large quantities of milk, which can only be maintained if forage diets are supplemented with concentrates. Supplementation of dairy cow diets with optimal amounts of energy substrates provide the high producing dairy cow with energy needed to improve fibre digestibility and utilization. Energy improves the total digestibility of a diet when forages are supplemented with energy sources, but decreases the utilization of the forage component *per se.* Possible reasons for the latter could be the structure of the fibre matrix, making it more difficult for rumen organisms to reach the more digestible fractions in forages, thereby digesting first the easily fermentable energy sources before attacking the more complex fibre fractions. It should be noted, however, that overfeeding of energy supplements increase the risks of acidosis, due to the production of large amounts of lactic acid, subsequently lowering milk production and income.

Looking at the individual forage fermentation kinetics, irrespective of the energy source used, it is evident that the pasture grasses had higher total gas production values than the straw and hays. This is most likely because the pasture grasses were cut after only 28 days of re-growth, whereas the hays and straw were more mature and of lower quality, especially the straw. The pasture grasses thus had more readily fermentable nutrients and less NDF and ADL than the hays and straw, leading to higher gas production values. The fermentation rate of the forages supplemented with energy sources differed amongst each other, with lucerne and ryegrass having the fastest fermentation rates, irrespective of the energy source supplemented. Gas production of forages supplemented with citrus pulp and maize meal were higher compared to molasses. Molasses produced a greater volume of gas during the first few hours of incubation, but it was also quickly depleted due to its high content of readily fermentable sugars. These sugars are highly soluble and rumen micro-organisms have easy access to induce fermentation. The gas production rate of forages supplemented with energy sources was higher than the control treatments (forages alone), with molasses and citrus pulp resulting in the highest rates. It thus seemed that supplementation of energy sources improved forage fermentability as well as the rate of forage fermentation. This could have major implications in practice as there is a need to find ways of improving fibre utilization in South Africa.

The effect of the energy sources on the fermentation kinetics of different forages *per se* showed a decrease in gas production and lag phase, but a tendency to raise gas production rate. Molasses decreased gas production the most throughout all the forage substrates. A possible reason may be that the rumen micro-organisms first digest the fast fermentable simple sugar substrates before starting with substrates that are digested at a slower rate. The raise in gas production rate and decrease in lag time may be due to a higher number of rumen micro-organisms available to ferment the feed, when energy sources were added to the forage substrates, thus supporting digestion.

Little research has been done on this and related topics to quantify the relationship between different carbohydrate sources and rumen metabolism parameters, leaving room for improvement and further studies. More research is also needed with regard to inclusion levels of different energy sources in lactating cow diets and the potential outcomes regarding milk production and rumen health.

3.6. References

Adesogan, T., Krueger, N.K. & Kim, S.C., 2005. A novel, wireless, automated system for measuring fermentation gas production kinetics of feeds and its application to feed characterization. Anim. Feed Sci. Technol. 123 - 124(1), 211 - 223.

Aldrich, J.M., Muller, L.D. & Varga, G.A., 1993. Nonstructural carbohydrates and protein effects on rumen fermentation, nutrient flow and performance of dairy cows. J. Dairy Sci. 76, 1091 - 1105.

Allen, M. & Oba, M., 2000. Getting more milk from forages. Michigan Dairy Review 5(4), Michigan State University.
Available at:
http://www.admani.com/alliancedairy/TechBulletins/Non%20Structural%20Carbohydrate%20Nutrition.htm
(Accessed 5 August 2008)

ANKOM, 2005. Method for determining acid detergent lignin in beakers, ANKOM *Technology,* 08/05.

AOAC, 1990. Official Methods of Analysis. (15th Ed.). Association of Official Analytical Chemists. Inc., Arlington, Virginia, USA.

AOAC, 1995. Official Methods of Analysis. (15th Ed.). Association of Official Analytical Chemists, Inc., Washington, D.C., USA.

Arigbede, O.M., Anele, U.Y., Olanite, J.A., Adekunle, I.O., Jolaosho, O.A. & Onifade, O.S., 2006. Seasonal *in vitro* gas production parameters of three multi - purpose tree species in Abeokuta, Nigeria. Livestock Res. Rural Dev. 18(10), Article 142.
Available at: http://www.cipav.org.co/lrrd/lrrd18/10/arig18142.htm
(Accessed 14 October 2008)

Bampidis, V.A. & Robinson, P.H., 2006. Citrus by-products as ruminant feeds: A review. Anim. Feed Sci. Technol. 128, 175 - 217.

Cheng, K.J., Stewart, C.S., Dinsdale, D. & Costeron, J.W., 1984. Electron bacteria involved in the digestion of plant cell walls. Anim. Feed Sci. Technol. 10, 93 - 120.

Chesson, A. & Forsberg, C. W., 1988. Polysaccharide degradation by rumen micro-organisms, In: The Rumen Microbial Ecosystem. Ed. Hobson, P.N., Elsevier Science Publishers Ltd. London, UK. pp. 251 - 284.

Ghadaki, M.B., Van Soest, P.J., McDowell, R.E. & Malekpour, B., 1975. Chemical composition and in vitro digestibility of some range forage species of Iran. In: Proceedings of the sem. Bamako, 2 - 3 March, Bamako, Mali.
Available at: http://www.fao.org/wairdocs/ilri/x5543b/x5543b0z.htm
(Accessed 17 October 2008)

Giraldo, L.A., Tejido, M.L., Ranilla, M.J. & Carro, M.D., 2008. Effect of exogenous fibrolytic enzymes on *in vitro* rumen fermentation of substrates with different forage:concentrate ratios. Anim. Feed Sci. Technol. 141, 306 - 325.

Goosen, L., 2004. Effect of an exogenous fibrolytic enzyme on forage digestibility parameters, MSc (Agric) thesis, Stellenbosch University, Stellenbosch, South Africa. pp. 1 - 91.

Haddad, S.G. & Grant, R.J., 2000. Influence of nonfiber carbohydrate concentration on forage fiber digestion *in vitro*. Anim. Feed Sci. Technol. 86, 107 - 115.

Hall, M.B., 2002. Working with sugars (and molasses). In: Proceedings of the 13th Annual Florida Ruminant Nutrition Symp., Jaunary 11 - 12, Gainesville, Florida, USA. pp. 146 - 158.

Hiltner, P. & Dehority, A., 1983. Effect of soluble carbohydrates on digestion of cellulose by pure cultures of rumen bacteria. Appl. Environ. Microbiol. 46, 542.

Larson, C.C., 2003. The effect of nonfibre carbohydrate source and protein degradability on lactation performance of holstein cows, MSc (Agric) thesis, University of Florida. Gainesville, Florida, USA. pp. 1 - 108.

Leiva, E., Hall, M.B. & Van Horn, H.H., 2000. Performance of dairy cattle fed citrus pulp or corn products as sources of neutral detergent-soluble carbohydrates. J. Dairy Sci. 83, 2866 - 2875.

Marais, J.P., Therion, J.J., Mackie, R.I., Kistner, A. & Dennison, C., 1988. Effect of nitrate and its reduction products on the growth and activity of the rumen microbial population. Brit. J. Nutr. 59, 301 - 313.

Mauricio, R.M., Mould, F.L., Dhanoa, M.S., Owen, E., Channa, K.S. & Theodorou, M.K., 1999. A semi-automated *in vitro* gas production technique for ruminants feedstuff evaluation. Anim. Feed Sci. Technol. 79, 321 - 330.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 2002. Evaluation of food: Digestibility. In: Animal Nutrition. (6th Ed.). Pearson Education Ltd. Edinburgh Gate, Harlow, Essex, UK. pp. 246 - 263.

Mertens, D.R., 1997. Creating a system for meeting the fibre requirements of dairy cows. J. Dairy Sci. 80, 1463 - 1481.

Mertens, D.R. & Loften, J.R., 1980. The effect of starch on forage fiber digestion kinetics *in vitro*. J. Dairy Sci. 63, 1437 - 1446.

Miller, B.G. & Muntifering, R.B., 1985. Effect of forage:concentrate on kinetics of forage fiber digestion *in vitro*. J Dairy Sci. 68, 40 - 44.

Mohney, K., 2002. Synchronization of carbohydrate and protein metabolism by ruminal microbes in continuous culture. PhD thesis, North Carolina State University, Raleigh, North Carolina, USA. pp. 1 - 69.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. (7th Rev. Ed.). National Academy. Press, Washington, D.C., USA. pp. 34 - 35.

Sallam, S.M.A., 2005. Nutritive value assessment of the alternative feed resources by gas production and rumen fermentation *in vitro*. Res. J. Agric. & Biol. Sci. 1(2), 200 - 209.

Schwarz, F.J., Haffner, J. & Krichgessner, M., 1995. Supplementation of zero-grazed dairy cows with molassed sugar beet pulp, maize or cereal-rich concentrate. Anim. Feed Sci. Technol. 54, 237 - 248.

Solomon, R., Chase, L.E., Ben-Ghedalia, D & Bauman, D.E., 2000. The effect of nonstructural carbohydrate and addition of full fat soybeans on the concentration of conjugated linoleic acid in milk fat of dairy cows. J. Dairy Sci. 83, 1322 - 1329.

Sommart, K., Parker, D.S., Wanapat M. & Rowlinson, P., 2000. Fermentation characteristics and microbial protein synthesis in an *in vitro* system using cassava, rice straw and dried ruzi grass as substrates. Asian-Aust. J. Anim. Sci., 13, 1084 - 1093.

Statistica 8.1., 2008. StatSoft Inc., USA.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant (2nd Ed.). Cornell University Press, Ithaca, New York, USA. pp. 251 - 252.

Van Soest, P.J. & Robertson, J.B., 1985. Analysis of forages and fibrous feeds. A laboratory manual for animal science 613. Cornell University, Ithaca, New York, USA.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583 - 3587.

Ørskov, E.R. & McDonald, P., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci. 92, 499 - 503.

Chapter 4

THE EFFECT OF SUGAR, STARCH AND PECTIN AS MICROBIAL ENERGY SOURCES ON *IN VITRO* NEUTRAL DETERGENT FIBRE AND DRY MATTER DEGRADABILITY OF FORAGES

Abstract

The study evaluated the effect of sugar (molasses and sucrose), starch (maize meal and maize starch) and pectin (citrus pulp and citrus pectin) on neutral detergent fibre (NDF) and dry matter (DM) degradability of forages. Forage substrates used included wheat straw (WS), oat hay (OH), lucerne hay (LUC), ryegrass (RYE) and kikuyu grass (KIK). Rumen fluid was collected from two lactating Holstein cows receiving a diet consisting of oat hay, lucerne, wheat straw and a concentrate mix. *In vitro* degradability was done with an ANKOM Daisy II incubator and forage substrates were incubated, with or without, the respective energy sources, for 24, 48 and 72 hours. The substrates were incubated in 1076 ml buffered medium, 54 ml of reducing solution and 270 ml rumen fluid. The residues were washed, dried and analyzed for NDF. In the trial with the applied energy sources (molasses, maize meal and citrus pulp), there was forage x energy source interactions. Supplementation with the applied energy sources all improved DMD of forages (24 & 72 hours), when compared to the control treatment, except for RYE supplemented with maize meal and citrus pulp at 24 hours. Molasses had the biggest effect on DMD in all forage substrates. Supplementation with maize meal had no effect on neutral detergent fibre degradability (NDFD) of any forage substrate, except for an improvement in NDFD of LUC at 72 hours. Molasses improved NDFD of LUC at 24 hours, but had no effect on the other forage substrates. Citrus pulp improved NDFD of OH (72 hours), as well as LUC and WS (24 & 72 hours). It is postulated that the NDF of the energy sources was more digestible than that of the respective forages, and that the improved NDFD values could be ascribed to the contribution of the energy source NDFD. Overall, pasture grasses had a higher NDFD than the hays and straw, and appear to be more readily fermentable by rumen micro-organisms than the low quality hays and straw, explaining the higher NDFD. In the trial involving the purified energy sources (sucrose, maize starch and citrus pectin), forage x energy source interactions were observed. In general, supplementation with these energy sources improved DMD at 24 and 72 hours except for RYE and KIK (72 hours). Pasture grasses (RYE & KIK) had a higher NDFD than LUC, OH and WS. At 72 hours, NDFD was 37.1% for LUC, 42.5% for OH and 40.3% for WS, compared to 70.5% for KIK and 64.9% for RYE. A possible explanation is that KIK and RYE samples came from freshly cut material, harvested after a 28d re-growth period. In general, sucrose (24 & 72 hours) and citrus pectin (72 hours) had no effect on NDFD of forage substrates. Supplementing oat hay (24 hours) with starch and citrus pectin, and wheat straw (24 & 72 hours) with starch, however, lowered NDFD ($P < 0.05$) when compared to the control treatment. It is hypothesized that micro-organisms fermented the easily fermentable energy sources first, before attacking forage NDF. The study suggested that forage NDFD values are not fixed, and may be altered by type of energy supplementation.

4.1. Introduction

The production potential of ruminants is determined to a great extent by the availability and quality of forages. Lactating dairy cows depend significantly on forages to maintain optimal fermentation, rumen function and high production. However, the ability of rumen micro-organisms to degrade forages is restricted by the chemical composition and physical quality of the forage (Mertens, 1997). Forages alone also do not provide all the energy requirements of a high producing dairy cow (Schwarz *et al.*, 1995). Supplementing dairy cow diets with energy-rich feeds provide high yielding dairy cows with the energy needed to improve efficiency of production and performance (Henning, 2004). The most important source of energy and largest nutrient component for rumen micro-organisms is carbohydrates (especially non-fibre carbohydrates or NFC).

It is important to try and find ways to improve forage degradation. Improvement of forage utilization and degradation will aid in animal performance (Giraldo *et al*, 2008). Forage based diets supplemented with NFC (sugar, starch or pectin) result in variation of performance measurements such as milk yield, milk composition, dry matter intake (DMI) and feed efficiency (Larson, 2003). Miron *et al.* (2002) reported that cows receiving total mixed rations (TMR) with a high percentage of citrus pulp had higher NDF and NSC digestibilities compared to cows that received TMR with a high percentage of corn. Leiva *et al.* (2000) reported that the rumen pH declined more rapidly on citrus diets (pectin) than on hominy (starch) diets, and also reached the lowest pH point faster. Knowledge of the individual (as well as a combination of NFC) fermentation characteristics can thus be helpful in predicting an animal's performance due to NFC supplementation (Holtshausen, 2004).

The objectives of this study were to determine the impact of three applied energy sources, *viz.* maize meal, molasses syrup and citrus pulp and three purified energy sources, *viz.* maize starch, sucrose and citrus pectin on dry matter (DM) and neutral detergent fibre (NDF) digestibility of different forage substrates. Forages commonly used in dairy cow diets (wheat straw, oat hay, lucerne hay, kikuyu grass and ryegrass) were used.

4.2. Materials and methods

4.2.1. Study area

The study to evaluate the effect of supplementing forage based diets with sugar, starch and pectin on DM and NDF degradability was conducted at the Stellenbosch University, Stellenbosch, South Africa (33° 55′ 12″ S, 18° 51′ 36″ E).

4.2.2. Simulated diets

4.2.2.1. Basal forages

Five forages (Table 4.1) were used to prepare rations to simulate diets for lactating dairy cows.

Table 4.1 Forages used in simulation diets for lactating dairy cows.

Forage Type	Acronym
Wheat straw (*Triticum aestivum*)	WS
Oat hay (*Avena sativa)*	OH
Lucerne hay (*Medicago sativa*)	LUC
Ryegrass (*Lolium multiflorum*)	RYE
Kikuyu grass (*Pennisetum clandestinum*)	KIK

Rye and kikuyu grasses were harvested after four weeks of re-growth. All the forages were oven dried at 60ºC for 72 hours. Wheat straw, oat hay and lucerne hay were ground (cyclotec 1093 mill) through a 2 mm screen. Rye and kikuyu grasses were obtained from Outeniqua experimental farm (33° 57′ 0″ S, 22° 25′ 0″ E) just outside George, South Africa (33° 58′ 0″ S, 22° 27′ 0″ E). The rye and kikuyu grasses were already ground through a 1 mm screen when received.

4.2.2.2. Energy supplements

Three energy sources (Table 4.2) were selected as supplements to prepare rations that would simulate lactating dairy cow diets.

Table 4.2 Applied energy sources used in simulating the dairy cow diets.

Energy type	Source	Acronym
Starch	Yellow maize (*Zea mays)* meal	Mm
Sugar	Molasses syrup	Mol
Pectin	Citrus pulp	Cp

These energy feedstuffs were sourced in the following forms: molasses as a syrup by-product from the processing of sugar cane (*Officinarum saccharum*); citrus pulp as a finely granulated residue by-product from the peel, pulp and seeds of oranges and grapefruit and yellow maize grain. The citrus pulp and maize were ground (cyclotec 1093 mill) through a 1 mm screen.

Three purified energy sources (Table 4.3) were selected as supplements to prepare rations that would simulate lactating cow diets.

Table 4.3 Purified energy sources used in simulating the dairy cow diets.

Energy type	Source	Acronym
Starch	Maize starch	Maiz
Sugar	Sucrose	Suc
Pectin	Citrus pectin	Pec

4.2.2.3. Defining the diets

A total of 43 simulated diets were prepared:

- 5 diets contained forage substrates only (Table 4.1).
- 3 diets contained applied energy sources only (Table 4.2).
- 15 diets contained a mixture of forages and applied energy sources.
- 5 diets contained forage substrates only (Table 4.1).
- 15 diets contained a mixture of forages and purified energy sources.

The final substrate compositions are indicated in Tables 4.5 and 4.6.

4.2.3. Chemical analyses of forages and energy sources

About 1 g of each forage type, as well as 1 g of the energy sources were weighed and placed in a 100ºC conventional oven for 24 hours to determine DM content (AOAC, 1995; Method 930.15). Organic matter (OM) was determined by weighing 1 g of each type of feedstuff used into crucibles and ashing the content at 500ºC in a muffle furnace for 6 hours (AOAC, 1995; Method 942.05).

The NDF component was determined by measuring 0.5 g of each feedstuff into F57 ANKOM fibre analysis bags. The bags were heat sealed and NDF determined using the method of Van Soest *et al*. (1991). Sodium sulfite (Na_2SO_3) was added to the NDF solution during digestion and heat-stable amylase was added during rinsing. Ether extract was determined using the AOAC method (AOAC, 1995; Method 920.39). About 2 g of ground sample was weighed into a thimble. The samples were then extracted with diethyl ether ($C_4H_{10}O$).

Acid detergent lignin (ADL) was determined by measuring 0.5 g of each basal forage and 0.5 g of maize meal and citrus pulp into separate F57 ANKOM fibre analysis bags. The bags were heat sealed and acid detergent fibre (ADF) was determined using the method of Van Soest *et al*. (1991). The ADF residue was then soaked in 72% sulphuric acid for three hours to dissolve the cellulose. Acid detergent lignin was determined using the ANKOM method (ANKOM, 2005).

Total nitrogen content was determined with a Leco Nitrogen Gas Analyzer custom designed and built by LECO Africa (Pty) Ltd (Kempton Park). About 0.1 g of sample was weighed into a small piece of aluminum foil. The samples were then ignited inside the Leco furnace at about 900°C using the Dumas procedure (AOAC, 1990; Method 968.06). Crude protein (CP) was calculated by multiplying nitrogen content with 6.25 (AOAC, 1995; Method 990.03).

Table 4.4 Chemical composition (g/kg DM ± SD) of forages and energy sources used in the trial. All values are on a DM basis.

Sources	DM	OM	NDF	CP	EE	ADL
WS	915.0±0.5	902.2±1.6	817.9±7.1	72.3±1.0	3.6±0.5	102.2±3.0
OH	850.4±2.3	940.3±17.3	781.9±11.0	91.0±1.2	9.7±0.8	82.5±0.3
LUC	898.9±2.0	906.2±1.8	515.3±1.0	237.0±4.6	8.1±0.6	92.7±6.7
RYE	93.8±1.0	84.5±0.5	489.2±3.0	250.9±2.7	43.1±0.1	44.8±18.1
KIK	124.2±10.4	112.2±0.1	651.2±2.0	253.4±4.1	28.3±1.1	48.0±1.7
mm	855.9±0.9	984.4±1.3	174.3±55.0	107.1±0.2	37.7±0.4	7.0±0.6
cp	879.9±0.9	928.5±0.7	261.4±14.0	78.6±1.8	16.4±0.4	25.3±6.4
mol	698.0±1.9	892.7±0.6	ND	ND	ND	ND

DM = dry matter; OM = organic matter; NDF = neutral detergent fibre; CP = crude protein; EE = ether extract; ADL = acid detergent lignin; ND = not determined; WS = wheat straw; OH = oat hay; LUC = lucerne; RYE = ryegrass; KIK = kikuyu grass; mm = maize meal; cp = citrus pulp; mol = molasses;
Purified energy sources were 99% pure, based on manufacturers analyses.

4.2.4. Sample preparation

Small fibre analysis bags were used (F57, ANKOM Technology). The bags were pre-rinsed in acetone for five minutes, air dried and placed in a conventional oven at 100ºC for three to four hours. The diets were constituted as shown in Tables 4.5, 4.6 and 4.7. The total amount of substrate was based on a forage to concentrate ratio of 50:50. The substrate amount of the energy sources in Table 4.5 was calculated to provide the same amount of metabolizable energy (ME) contained in 0.25 g DM of yellow maize meal. For calculation purposes the ME values were assumed to be 13.9 MJ/kg DM for maize, 12.3 MJ/kg for molasses and 12.2 MJ/kg for citrus pulp (NRC, 2001).

Table 4.5 Substrate samples containing either forage or applied energy supplements.

Forage type	Energy source	Forage (g DM)	Energy (g DM)
Wheat straw	-	0.250	-
Oat hay	-	0.250	-
Lucerne hay	-	0.250	-
Ryegrass	-	0.250	-
Kikuyu grass	-	0.250	-
-	Maize meal	-	0.250
-	Citrus pulp	-	0.285
-	Molasses	-	0.282

The amount of energy sources used in the composite substrates (Table 4.6) was calculated to provide the same amount of ME contained in 0.125 g DM of yellow maize meal (13.9 MJME/kg).

Table 4.6 Composite diets containing forage and applied energy sources.

Forage type	Energy source	Forage (g DM)	Energy (g DM)
Wheat straw	Maize meal	0.1250	0.1250
Wheat straw	Citrus pulp	0.1250	0.1425
Wheat straw	Molasses	0.1250	0.1412
Oat hay	Maize meal	0.1250	0.1250
Oat hay	Citrus pulp	0.1250	0.1425
Oat hay	Molasses	0.1250	0.1412
Lucerne hay	Maize meal	0.1250	0.1250
Lucerne hay	Citrus pulp	0.1250	0.1425
Lucerne hay	Molasses	0.1250	0.1412
Ryegrass	Maize meal	0.1250	0.1250
Ryegrass	Citrus pulp	0.1250	0.1425
Ryegrass	Molasses	0.1250	0.1412
Kikuyu grass	Maize meal	0.1250	0.1250
Kikuyu grass	Citrus pulp	0.1250	0.1425
Kikuyu grass	Molasses	0.1250	0.1412

The amount of purified energy sources (Table 4.7) was based on suggestions by Holtshausen (2004) who used starch, pectin and sucrose at hexose equivalent amounts of 40, 80 or 120 mg per 120 mg purified NDF in *in vitro* incubations.

Table 4.7 Diets containing forages alone or a mixture of forages and purified energy sources. Amounts are on an air dry basis.

Forage type	Energy source	Forage (g)	Energy (g)
Wheat straw	-	0.500	-
Oat hay	-	0.500	-
Lucerne hay	-	0.500	-
Ryegrass	-	0.500	-
Kikuyu grass	-	0.500	-
Wheat straw	Maize starch	0.500	0.163
Wheat straw	Citrus pectin	0.500	0.192
Wheat straw	Sucrose	0.500	0.137
Oat hay	Maize starch	0.500	0.152
Oat hay	Citrus pectin	0.500	0.178
Oat hay	Sucrose	0.500	0.127
Lucerne hay	Maize starch	0.500	0.160
Lucerne hay	Citrus pectin	0.500	0.189
Lucerne hay	Sucrose	0.500	0.135
Ryegrass	Maize starch	0.500	0.162
Ryegrass	Citrus pectin	0.500	0.191
Ryegrass	Sucrose	0.500	0.137
Kikuyu grass	Maize starch	0.500	0.161
Kikuyu grass	Citrus pectin	0.500	0.190
Kikuyu grass	Sucrose	0.500	0.136

In the current study, a hexose equivalent amount of 40 mg of each energy source per 120 mg of forage substrate was used. Since 500 mg (air dry) of each forage substrate was weighed out into the incubation bags in the current study, the hexose equivalent of the energy sources amounted to 167 mg DM.

To calculate the actual amount of energy source DM, the following conversion coefficients were used (based on Holtshausen, 2004):

- 0.90 for maize starch
- 1.14 for citrus pectin
- 0.95 for sucrose

The DM content of the energy sources were taken into account when calculating the air dry amounts that were finally weighed out into the bags (Table 4.7).

Blank bags were included to correct for residues from rumen fluid alone. All bags were heat-sealed using an impulse sealer after sample preparation.

4.2.5. Preparation of *in vitro* medium and reducing solution

The incubation medium was prepared as described by Van Soest & Robertson (1985). The medium consisted of micro minerals, macro-mineral solution, buffer solution, tryptose, rezasurin and distilled water. The medium was kept in a water bath at 39.5ºC. The pH of the medium was about 7.8. Reducing solution was prepared as described by Van Soest & Robertson (1985) and consisted of cysteine hydrochloride ($C_3H_7NO_2 \cdot HCL$), potassium hydroxide (KOH) pellets, sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) and distilled water.

4.2.6. Collection and preparation of rumen fluid

Rumen fluid was collected from two ruminally cannulated lactating dairy cows. The cows were confined and fed 25 kg (as is) of a diet (12.5 kg in the mornings and 12.5 kg in the evenings) consisting of oat hay, lucerne hay, wheat straw and a concentrate mix. Rumen fluid was squeezed through two layers of cheesecloth into pre-warmed flasks and a small amount of coarse material was added. The rumen fluid was then blended in a pre-warmed blender at a low speed for 10 seconds. The rumen fluid was then filtered through four layers of cheesecloth into pre-warmed flasks while flushing with carbon dioxide (CO_2). The temperature of the rumen fluid averaged 38ºC and the pH averaged 5.8.

4.2.7. *In vitro* incubation

The *in vitro* procedure for the ANKOM Daisy incubations required four glass vessels (2 L) and is based on the method described by Goering & Van Soest (1970). The glass vessels were flushed with CO_2 while adding 1076 ml of incubation medium and 54 ml of reducing solution to each vessel. The vessels were closed and placed in the incubator at 39.5ºC until the medium was clear. The vessels were opened and flushed with CO_2 while adding 270 ml of rumen fluid and bags to the vessels. Incubation periods were 24 and 72 hours. At each time interval one vessel was removed and the residue bags washed with water, air-dried and then placed in a conventional oven at 100ºC for 24 hours.

4.2.8. Residue analysis

The NDF content of the residues in the bags was determined using an ANKOM fibre machine (ANKOM^{220} fibre analyzer) and was based on the method of Van Soest *et al*. (1991). Sodium sulfite (20 g) was added to 1.9 L of NDF solution during digestion and heat-stable amylase (4 ml x 2 rinse) was added during rinsing. Before using the bags, they were rinsed in acetone for three to five minutes and then air-dried before being placed in a forced draft oven at 100ºC for 24 hours. After the NDF procedure, the bags were dried, weighed and subsequently ashed in a muffle furnace at 500ºC for 6 hours.

4.2.9. Estimating dry matter degradability

The estimation of DM degradability of the substrates was based on DM disappearance and calculated in Excel using the following equation (Van Soest *et al*., 1991):

$$DMD = 100 - \frac{[W_3 - (W_1 \times C_1)]}{W_2} \times 100 \qquad \text{Equation 5}$$

Where:	DMD	=	Apparent DM degradability (%)
	W_1	=	Bag weight (mg)
	W_2	=	Sample weight (mg DM)
	W_3	=	Weight of dried bag and residue after incubation (mg DM)
	C_1	=	Blank bag correction factor

4.2.10. Estimating neutral detergent fibre degradability

The estimation of NDF% degradability of the substrates was based on NDF disappearance and was calculated in Excel using the following equation (Van Soest *et al*., 1991):

$$NDF = 100 - \frac{[W_3 - (W_1 \times C_1)]}{W_2} \times 100 \qquad \text{Equation 6}$$

Where:	NDF	=	Apparent NDF degradability (%)
	W_1	=	Bag weight (mg)
	W_2	=	Sample weight (mg NDF in DM)
	W_3	=	Dried weight of bag with residue after incubation (mg NDF in DM)
	C_1	=	Blank bag correction factor

4.3. Statistical analyses

The *in vitro* digestibility experiment was a three way cross classification and the data was subjected to a factorial ANOVA with the three factors being forage, energy and time using Statistica 8.1 (2008). This was done for all the non-linear parameters. Significant forage x energy interactions were observed for the non-linear parameters. Therefore, a one-way ANOVA was done on each of the forages to determine the effect of the energy sources. Differences between means were determined using a Tukey test. Significance was declared at $P < 0.05$.

4.4. Results and discussion

4.4.1. Effect of maize meal, citrus pulp and molasses on apparent *in vitro* dry matter degradability

Results of the *in vitro* dry matter degradability (DMD) trial are presented in Table 4.8. Unsupplemented forage values clearly indicate that ryegrass and kikuyu are more digestible than lucerne hay, oat hay and wheat straw. This can be attributed to the fact that pasture grasses were cut after only 28 days of re-growth, thus having more digestible nutrients compared to lucerne and oat hay which were harvested at the 10% flowering stage and wheat straw at maturity. With maturity, the NDF and ADL contents increase (Table 4.4) and DMD decreases (Canbolat *et al*., 2006). When forage substrates were supplemented with applied energy sources, a forage x energy source interaction was observed. When maize meal, citrus pulp and molasses replaced 50% of the forage substrates, DMD was increased ($P < 0.01$) in all forage substrates (after 24 and 72 hours of incubation), with molasses having the biggest effect throughout. It should be kept in mind that the energy sources as such were all more degradable than the forages, and therefore it could be expected that a 50% replacement of forages with energy sources would increase total substrate DMD. The increase in DMD of forage substrates could thus be due to increased stimulation of rumen micro-organisms in response to the readily available energy supplied by energy sources.

Table 4.8 Effects of maize meal, citrus pulp and molasses as energy sources on *in vitro* dry matter degradability (DMD) parameters of different forage substrates.

	Treatment					
Item	**Maize meal**	**Citrus pulp**	**Molasses**	**Control**	**SEm**	**P**
Lucerne hay						
DMD 24h	70.7 [b]	73.1 [b]	83.2 [c]	56.5 [a]	1.59	<0.001
DMD 72h	82.9 [b]	82.5 [b]	85.8 [c]	69.0 [a]	0.44	<0.001
Oat hay						
DMD 24h	63.7 [b]	67.6 [bc]	73.9 [c]	44.2 [a]	1.53	<0.001
DMD 72h	77.3 [b]	78.8 [bc]	82.0 [c]	58.8 [a]	0.80	<0.001
Wheat straw						
DMD 24h	60.2 [b]	62.7 [b]	71.0 [c]	38.3 [a]	0.92	<0.001
DMD 72h	72.1 [b]	74.4 [bc]	77.8 [c]	51.0 [a]	0.89	<0.001
Ryegrass						
DMD 24h	79.6 [a]	83.5 [ab]	90.1 [b]	76.0 [a]	2.12	0.003
DMD 72h	92.6 [b]	93.4 [b]	96.4 [b]	87.7 [a]	0.95	<0.001
Kikuyu grass						
DMD 24h	80.2 [b]	80.6 [b]	88.6 [c]	69.5 [a]	1.88	<0.001
DMD 72h	91.5 [b]	92.3 [b]	95.3 [b]	86.7 [a]	0.98	<0.001

Superscripts are compared across rows.

The biggest effect of energy supplementation occurred with wheat straw, as also reported in the gas production experiments (Chapter 3). Wheat straw was high in NDF and ADL (Table 4.4). Neutral detergent fibre is slowly degraded by rumen micro-organisms, whereas ADL are not degraded by rumen micro-organisms explaining the lower DMD of wheat straw when compared to the other forages. Supplementing readily available energy sources would therefore have a greater impact on DMD of wheat straw compared to other forages. Molasses had the highest impact on wheat straw DMD during the early phases of incubation (24 hours), but after 72 hours, the effect of molasses and citrus pulp was similar.

In lucerne hay, molasses had the highest impact on DMD at 24 and 72 hours incubation, but citrus pulp and maize meal also improved DMD significantly. In oat hay, maize meal, citrus pulp and molasses improved DMD at 24 and 72 hours incubation, with molasses also having the most noticeable effect ($P < 0.01$). Fall *et al.* (1998) also found improvements in DMD of straws supplemented with molasses. In contrast to results obtained in this study, Williams *et al.* (1953) found that addition of starch to oat hay diets of sheep decreased DMD by one to two percentage units. Burroughs *et al*. (1949) observed a 5 – 12% reduction in lucerne hay DMD when the diet was supplemented with 60% starch. Mould & Orskov (1983), however, concluded that

the decrease in DMD of hays are mainly due to reduced rumen pH and not directly related to starch digestion. Abrupt changes in cattle diets (forage to concentrate diets) decrease rumen pH, resulting in an overgrowth of starch fermenting bacteria which causes acute acidosis, with devastating effects on production (Owens *et al.,* 1998). Cellulolytic bacteria are also inhibited at low rumen pH values, decreasing fibre digestion (Russel & Wilson, 1996).

In ryegrass, molasses was the only energy source that improved DMD after 24 hours incubation. Simeone *et al.* (2004) found similar results and reported that neither ground nor whole maize meal had an effect on ryegrass DMD. Ryegrass and kikuyu are highly fermentable forages, as was also observed in terms of total gas production and rate of gas production (Chapter 3). Molasses, which is also rapidly fermentable, had the largest influence on DMD of pasture grasses at 24 hours, probably because of its high fermentation rates (Chapter 3) and because it is free of NDF and does not have trace amounts of lignin (Table 4.4). Overall however, all the energy sources improved DMD of ryegrass and kikuyu to the same extent at 72 hours.

4.4.2. Effect of maize meal, citrus pulp and molasses on apparent *in vitro* neutral detergent fibre degradability

The effects of sugar (molasses), starch (maize meal) and pectin (citrus pulp) on *in vitro* apparent NDF degradability (NDFD) of different forages and forage-energy source combinations are shown in Table 4.9. Unsupplemented pasture grasses harvested at 28 days of re-growth had a higher NDFD than the mature forages. The NDFD of mature forages ranged between 19.7 and 28.4%, which is too low to sustain production and hence the need for energy supplementation. Ryegrass and kikuyu was lower in NDF and ADL (Table 4.4) and had greater gas production volumes (above 320 ml/g OM; Chapter 3) compared to the other forages (ranging between 229 and 297 ml/g OM). A negative correlation between ADL content and NDFD of forage substrates have been reported by Smith *et al*. (1972) and Tomlin *et al.* (1964).

When forage substrates were supplemented with applied energy sources, a forage x energy source interaction was observed. When energy sources replaced 50% of the forage substrates, 24 hours NDFD of lucerne hay was significantly increased by citrus pulp and molasses. Hall (2002a), however, postulated that molasses decreases fibre digestion when fed in large quantities. After 72 hours, both maize meal and citrus pulp resulted in increased NDFD. Contrary to this observation, Mertens & Loften (1980) reported that starch decreased the potential extent of fibre digestion. These results (Mertens & Loften, 1980), however, confirm our findings obtained in the gas production experiments (Chapter 3), namely that maize meal tended to increase the lag time of lucerne hay. The lack of response of molasses at 72 hours could be due to its rapid fermentation rate (Chapter 3) and also to the fact that molasses does not have any NDF (Table 4.4) that could contribute to NDFD. Maize meal and especially citrus pulp, however, contain some NDF that is readily digestible.

Table 4.9 Effects of maize meal, citrus pulp and molasses as energy sources on *in vitro* neutral detergent fibre degradability (NDFD) parameters when incubated in combination with different forage substrates.

	Treatment					
Item	**Maize meal**	**Citrus pulp**	**Molasses**	**Control**	**SEm**	**P**
Lucerne hay						
NDFD 24h	23.0 [ab]	29.6 [b]	30.0 [b]	19.7 [a]	2.19	0.014
NDFD 72h	50.3 [b]	54.1 [b]	41.4 [a]	40.3 [a]	1.26	<0.001
Oat hay						
NDFD 24h	30.0	35.6	28.9	28.4	2.22	0.138
NDFD 72h	52.2 [ab]	57.9 [b]	50.9 [a]	47.2 [a]	1.39	<0.001
Wheat straw						
NDFD 24h	19.6 [a]	28.5 [b]	24.9 [ab]	24.7 [ab]	1.71	0.024
NDFD 72h	43.7 [a]	50.9 [b]	42.1 [a]	40.3 [a]	1.63	0.003
Ryegrass						
NDFD 24h	52.2	59.9	57.1	50.1	2.49	0.059
NDFD 72h	77.5	82.2	84.3	74.8	2.42	0.063
Kikuyu grass						
NDFD 24h	51.6	56.1	62.4	53.0	4.24	0.326
NDFD 72h	79.4	82.6	83.7	79.5	2.05	0.368

Superscripts are compared across rows.

Apparent NDFD of oat hay was not affected by energy source after 24 hours incubation, but with citrus pulp, longer incubations (72 hours) resulted in higher NDFD ($P < 0.01$), probably due to a relative high digestibility of citrus pulp NDF. Miron *et al.* (2002) obtained similar positive results on NDFD of forages when maize or barley was replaced with citrus pulp. Voelker & Allen (2003) proposed that the optimal rate and extent of NDFD of high concentrate diets can be achieved by using dried beet pulp instead of high moisture corn. Heldt *et al.* (1999) found that supplementing low-quality tallgrass-prairie hay with degradable intake protein and NFC increased the total tract NDFD, but supplementation with NFC alone decreased NDFD.

Citrus pulp was the only energy source that consistently improved apparent NDFD when incubated in combination with the hays and straw. Citrus pulp had significant positive effects on NDFD of these forages, probably because mature forages are slowly degradable and citrus pulp supplementation thus provided high amounts of digestable fibre at both 24 hours and 72 hours incubations. The degradation rate of citrus pulp possibly synchronizes the release of nutrients with the requirements of fibrolytic bacteria. Increased NDFD is important as it increases the supply of energy and support microbial N production (Allen & Oba, 2000),

resulting in higher milk production and reduced rumen fill in lactating dairy cows (Dado & Allen, 1995). Bampidis & Robinson (2006) stated that citrus pulp might be a good supplement to dairy cow diets as it improves NDFD and has less negative effects on rumen pH and cellulolytic activities, compared to sugar and starch.

Energy sources had no effect on apparent NDFD of kikuyu and ryegrass, possibly because these pasture grasses was immature and thus high in soluble, readily available energy. In the gas production experiments (Chapter 3), these pasture grasses also had higher gas production volumes than the mature forages. It would thus appear, given the high nutrient content in these forages, that energy supplementation would have minimal effects on NDFD.

None of the energy source and forage combinations had lower NDFD values than the forages alone, contrary to Sanson *et al.* (1990) who reported that the supplementation of low-quality forages with rapidly degradable NFC depressed NDFD. Grant & Mertens (1992) suggested that acidification of the rumen, due to fast ruminal NFC fermentation, is a prime factor responsible for depression in fibre digestion.

4.4.3. Effect of maize starch, citrus pectin and sucrose on apparent *in vitro* dry matter degradability

The effect of purified energy sources, *viz*. sugar (sucrose), starch (maize starch) and pectin (citrus pectin) on *in vitro* apparent DMD of different forages are shown in Table 4.10. When forage substrates were supplemented with purified energy sources, a forage x energy source interaction was observed. In lucerne hay, only pectin resulted in an improved DMD (both at 24 hours and 72 hours), while in oat hay, all the energy sources improved DMD at both incubation times. Carey *et al.* (1993) reported similar improvements in DMD of brome hay (*in situ*) when it was supplemented with maize and beet pulp. In contrast, Burroughs *et al.* (1949) reported that DMD of lucerne was reduced when forages were supplemented with 20 and 40% starch. In wheat straw, all the energy sources improved DMD, but at 24 hours pectin and sucrose had a bigger effect than starch. Pectin and sucrose are more fermentable than starch, hence the more visible effect on DMD of slow degradable wheat straw forages. At 72 hours, pectin also improved DMD more than starch.

Ryegrass and kikuyu had high DMD values without supplementation. The higher DMD of pasture grasses was most likely because they were immature at harvesting, whereas lucerne and oat hay were cut at the 10% flowering stage and wheat straw was harvested at maturity. Immature pasture grasses were thus higher in fast fermentable sugars and lower in NDF and ADL (Table 4.4) improving DMD and gas production volumes (Chapter 3). Only sucrose was sufficient in improving DMD in these grasses and then only at 24 hours due to their very high fermentability. The absence of a sucrose effect at 72 hours was probably because sucrose was depleted before 72 hours, as it is rapidly fermented in the early hours of incubation. Purified energy sources are all more degradable than forages and therefore it could be expected that inclusion of these energy sources in forage diets will increase DMD. In general, purified energy sources resulted in lower DMD when compared to the applied energy sources (Table 4.8). We can postulate that

there was a rapid decline in rumen pH with purified energy source supplementation (as these energy sources has no NDF), resulting in higher lactic acid production and slower microbial growth, hence the lower DMD.

Table 4.10 Effects of maize starch, citrus pectin and sucrose as energy sources on *in vitro* dry matter degradability (DMD) parameters of different forage substrates.

	Treatment					
Item	**Maize starch**	**Citrus pectin**	**Sucrose**	**Control**	**SEm**	**P**
Lucerne hay						
DMD 24h	64.2 ^{ab}	68.7 ^{b}	66.0 ^{ab}	59.3 ^{a}	1.82	0.021
DMD 72h	74.5 ^{ab}	77.3 ^{b}	74.6 ^{ab}	67.3 ^{a}	2.03	0.025
Oat hay						
DMD 24h	47.6 ^{b}	51.2 ^{b}	50.4 ^{b}	37.7 ^{a}	0.96	<0.001
DMD 72h	63.8 ^{b}	65.8 ^{b}	64.4 ^{b}	55.2 ^{a}	1.16	<0.001
Wheat straw						
DMD 24h	44.9 ^{b}	50.8 ^{c}	52.0 ^{c}	34.8 ^{a}	0.80	<0.001
DMD 72h	59.1 ^{b}	64.3 ^{c}	61.7 ^{bc}	51.0 ^{a}	0.94	<0.001
Ryegrass						
DMD 24h	76.9 ^{ab}	76.8 ^{ab}	77.1 ^{b}	70.8 ^{a}	1.47	0.026
DMD 72h	88.4	86.2	87.5	84.7	1.85	0.548
Kikuyu grass						
DMD 24h	68.1 ^{ab}	68.4 ^{ab}	70.9 ^{b}	63.4 ^{a}	1.50	0.027
DMD 72h	85.9	82.6	84.3	80.8	2.13	0.408

Superscripts are compared across rows.

4.4.4. Effect of maize starch, citrus pectin and sucrose on apparent *in vitro* neutral detergent fibre degradability

Purified energy source supplementation on *in vitro* apparent NDFD of the different forage substrates are illustrated in Table 4.11. As was the case with DMD, an energy source x forage interaction was observed for NDFD. Purified energy sources have negligible amounts of NDF, thus the NDFD was solely from forages. In the control diets, it is evident that the immature pasture grasses had the highest NDFD, probably as they are low in NDF and ADL (Table 4.4).

With lucerne hay, energy supplementation had no significant effect on NDFD, while in oat hay, starch and pectin supplementation suppressed NDFD, but only at 24 hours incubation (P = 0.011). Sucrose had no

effect on NDFD of oat hay at 24 hours. Hall (2002a) proposed that, in general, sucrose tends to decrease fibre digestion when fed in large quantities, probably due to its lowering effect on rumen pH. Heldt *et al*. (1999) reported that NFC supplementation to low-quality tallgrass-prairie decrease NDFD, agreeing with our results.

Table 4.11 Effects of maize starch, citrus pectin and sucrose as energy sources on *in vitro* neutral detergent fibre degradability (NDFD) parameters of different forage substrates.

	Treatment					
Item	**Maize starch**	**Citrus pectin**	**Sucrose**	**Control**	**SEm**	**P**
Lucerne hay						
NDFD 24h	12.3	19.7	17.8	17.9	2.77	0.305
NDFD 72h	35.3	31.9	36.6	37.1	2.18	0.364
Oat hay						
NDFD 24h	13.1 ^{b}	13.7 ^{b}	17.4 ^{ab}	20.2 ^{a}	1.38	0.011
NDFD 72h	38.9	39.5	40.6	42.5	2.01	0.608
Wheat straw						
NDFD 24h	11.6 ^{b}	17.1 ^{a}	23.9 ^{c}	20.5 ^{ac}	1.26	<0.001
NDFD 72h	34.3 ^{b}	40.2 ^{a}	39.3 ^{a}	40.3 ^{a}	1.40	0.032
Ryegrass						
NDFD 24h	41.9	40.1	39.2	40.3	1.86	0.781
NDFD 72h	68.7 ^{a}	55.6 ^{b}	62.0 ^{ab}	64.9 ^{ab}	2.36	0.013
Kikuyu grass						
NDFD 24h	35.3	32.9	41.9	43.7	2.93	0.068
NDFD 72h	71.4 ^{a}	57.3 ^{b}	68.7 ^{ab}	70.5 ^{ab}	3.24	0.033

Superscripts are compared across rows.

Sucrose had no effect on NDFD of wheat straw, while pectin had a negative effect and starch severely suppressed NDFD at both incubation times ($P < 0.05$). The severe suppression in NDFD after supplementation of maize starch can result from the negative effect that starch has on rumen pH. Sucrose and starch ferment rapidly, producing lactic acid, a product that lowers rumen pH, whereas pectin does not produce lactic acid (Hall, 2002b). Starch has shown to decrease rumen pH and affect cellulolytic activity in the rumen, thereby lowering fibre digestion and increasing the risks of acidosis (Mertens & Loften, 1980). Heldt *et al*. (1999) reported similar results and found that starch decreased fibre digestion more than sugar. This is also in agreement with Vallimont *et al.* (2004) who found that by replacing starch with sugar incrementally in a continuous culture system, fibre digestion was increased.

Ryegrass and kikuyu NDFD was not affected at 24 hours by energy supplementation. Pasture grasses are high in rapidly fermentable sugars, especially when harvested young, as was the case with ryegrass and kikuyu (28 days re-growth). Supplementation with energy sources would thus have a much smaller effect on these forages compared to mature forages.

4.5. Conclusion

Forage based diets that constitute mainly of mature forages are deficient in energy and therefore cannot support growth and production in ruminant animals. Lactating dairy cows have high energy requirements for milk production. Supplementation of lactating dairy cow diets with concentrates, which are high in energy, can thus not be over-emphasized. Supplementation with optimal amounts of energy substrates provide the high producing dairy cow with energy needed to improve fibre digestibility and utilization. It must, however be kept in mind that incorrect feeding and large amounts of energy substrates (especially starch and sugar) increase the risks of ruminal acidosis due to the production of large quantities of lactic acid, which subsequently reduces milk production and profitability.

Results obtained from this study proved that supplementation of applied, as well as purified, energy sources have positive effects on DMD of forages. Supplementing forages with 50% of the applied energy sources improved DMD of all forages significantly. Purified energy sources improved DMD of the hays and straws significantly. These forages are mature and thus have less soluble, readily fermentable energy sources in their tissue compared to ryegrass and kikuyu. With the applied energy sources, molasses tended to have the biggest effect on DMD. In general, however, the applied energy sources had a greater influence on DMD, probably because they do not tend to lower rumen pH as much as purified energy sources.

The effect of applied energy sources on NDFD showed similar positive results. The energy sources improved NDFD of the total hay and straw substrates significantly. These forages are high in NDF and ADL which lowers NDFD. It must also be kept in mind that these energy sources also contain various amounts of NDF. Subsequently, substitution with energy sources (low in NDF and ADL) lowered the cell wall content of the substrate, thereby improving NDFD. Citrus pulp had the greatest effect on all forages NDFD, probably due to its highly digestible NDF content. The energy sources, however, had no effect on kikuyu grass and ryegrass, because these grasses were lower in NDF and ADL content and high in available sugars. Purified energy sources, in contrast, tended to decrease NDFD of all the forages. The negative effect on NDFD when supplementing with purified energy sources may be because fermentation of these energy sources by rumen micro-organisms, may result in a depletion of available nitrogen, needed by micro-organisms to ferment the slowly digestible fibre in forages (Heldt *et al.*, 1999). Another reason may be due to the fact that these energy sources lower the rumen fluid pH due to large amounts of lactic acid production (especially by sucrose and starch), thus decreasing the activity of cellulolytic and hemicellulolytic enzymes needed for fibre digestion (Hoover & Miller-Webster, 1998). Another reason may be that rumen micro-organisms first attack the readily fermentable energy sources before attaching to the forage substrates, thereby decreasing the rate of NDFD.

It must be emphasized that, when supplementing dairy cow diets with large amounts of energy sources, it is of utmost importance to provide adequate amounts of effective fibre to ensure a healthy rumen, as well as enough RDP to provide in the rumen microorganism's nitrogen requirements. *In vitro* experiments that show the effects of supplementing poor quality roughage (such as wheat straw) with RDP or non-protein nitrogen (NPN would also be useful in further studies, as the limiting nutrient in wheat straw is nitrogen. Nitrogen in turn is needed for energy-nitrogen coupling to take place and improve microbial growth. Not much research has also been done to quantify the relationship between different carbohydrate sources and rumen metabolism parameters, leaving room for improvement and further studies.

4.6. References

Allen, M. & Oba, M., 2000. Getting more milk from forages. Michigan Dairy Review 5(4), Michigan State University.
Available at:
http://www.admani.com/alliancedairy/TechBulletins/Non%20Structural%20Carbohydrate%20Nutrition.htm
(Accessed 5 August 2008)

ANKOM, 2005. Method for Determining Acid Detergent Lignin in Beakers, ANKOM *Technology,* 08/05.

AOAC, 1990. Official Methods of Analysis. 15th edition. Association of Official Analytical Chemists. Arlington, Virginia.

AOAC, 1995. Official Methods of Analysis. 15th edition. Association of Official Analytical Chemists, Washington, D.C., USA.

Bampidis, V.A. & Robinson, P.H., 2006. Citrus by-products as ruminant feeds: A review. Anim. Feed Sci. Technol. 128, 175 - 217.

Burroughs, W., Gerlaugh, W.P., Edington, B.H. & Bethke, R.M., 1949. The influence of corn starch upon roughage digestion in cattle. J. Anim. Sci. 8, 271.

Canbolat, O., Kamalak, A., Ozkan, C.O., Erol, A., Sahin, M., Karakas, E. & Ozkose, E., 2006. Prediction of relative feed value of alfalfa hays harvested at different maturity stages using *in vitro* gas production. Livestock Res. Rural Dev. 18(2), Article 27.
Available at: http://www.cipav.org.co/lrrd/lrrd18/2/canb18027.htm
(Accessed 18 October 2008)

Carey, D. A., Caton, J. S. & Biondini, M., 1993. Influence of energy source on forage intake, digestibility, in situ forage degradation and ruminal fermentation in beef steers fed medium-quality brome hay. J. Anim. Sci. 71, 2260 - 2269.

Dado, R.G. & M. S. Allen., 1995. Intake limitations, feeding behaviour, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. J. Dairy Sci. 78, 118 - 133.

Fall, S.T., Cissé, M., Ditaroh, D., Richard, D., Ndiaye, N.S. & Diaw, B., 1998**.** *In vivo* nutrient digestibility in sheep, and rumen dry matter degradability in cattle fed crop by-product based diets. J. Anim. Feed Sci. 7, 171 - 185.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analysis (apparatus, reagents, procedures and some applications). In: Agriculture Handbook No. 379. Agriculture Research Service, United States Department of Agriculture, Washington, USA.

Giraldo, L.A., Tejido, M.L., Ranilla, M.J. & Carro, M.D., 2008. In press: Effect of exogenous fibrolytic enzymes on *in vitro* rumen fermentation of substrates with different forage: concentrate ratios. Anim. Feed Sci. Technol. 141, 306 - 325.

Grant, R.J. & Mertens, D.R., 1992. Development of buffer systems for pH control and evaluation of pH effects on fiber digestion in vitro. J. Dairy Sci. 75, 1581 - 1587.

Hall MB. 2002a. Working with Non-NDF carbohydrates with manure evaluation and environmental considerations. In Proc. Mid-South Ruminant Nutrition Conference, Texas A & M University, Texas, USA. pp. 37 - 48.
Available at: http://www.txanc.org/proceedings/2002/Non-NDF%20Carbohydrates.pdf
(Accessed 20 October 2008)

Hall, M.B., 2002b. Working with sugars (and molasses). In: Proceedings of the 13th Annual Florida Ruminant Nutrition Symp., January 11 - 12, Gainesville, Florida, USA. pp. 146 - 158.

Heldt, J.S., Cochran, G.L., Stokka, G.L., Farmer, C.G., Mathis, C.P., Titgemeyer, E.C. & Nagaraja, T.G., 1999. Effects of different supplemental sugars and starch fed in combination with degradable intake protein on low-quality forage use by beef steers. J. Anim. Sci. 77, 2793 - 2802.

Henning, P., 2004. Acidosis in high-producing ruminants - myth or menace? Animal Feed Manufacturers Association (AFMA), South Africa, pp. 1 - 9.
Available at: http://www.engormix.com/e_articles_view.asp?art=529&AREA=GDC
(Accessed 5 Augustus 2008)

Holtshausen, L., 2004. Effect of nonfibre carbohydrates on product yield and fibre digestion in fermentations with mixed ruminal microbes. PhD thesis, University of Florida, Gainesville, Florida, USA. pp. 1 - 33.

Hoover, W.H. & Miller-Webster, T.K., 1998. Role of sugar and starch in ruminal fermentation. Tri-State Dairy Nutrition Conference, Michigan State University, pp. 1 - 16.

Larson, C.C., 2003. The effect of nonfiber carbohydrate source and protein degradability on lactation performance of Holstein cows, pp 1 - 108. MSc (Agric) thesis, University of Florida, Gainesville, Florida, USA. pp. 1 - 108.

Leiva, E., Hall, M.B. & Van Horn, H.H., 2000. Performance of dairy cattle fed citrus pulp or corn products as sources of neutral detergent-soluble carbohydrates. J. Dairy Sci. 83, 2866 - 2875.

Mertens, D.R., 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80, 1463 - 1481.

Mertens, D.R. & Loften, J.R., 1980. The effect of starch on forage fiber digestion kinetics *in vitro*. J. Dairy Sci. 63, 1437 - 1446.

Miron, J., Yosef, E., Ben-Ghedalia, D., Chase, L.E., Bauman, D.E. & Solomon, R., 2002. Digestibility by dairy cows of monosaccharide constituents in total mixed rations containing citrus pulp. J. Dairy Sci. 85, 89 - 94.

Mould, F. L. & Orskov, E. R., 1983. Manipulation of rumen fluid pH and its influence on cellulolysis *in sacco*, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. Anim. Feed Sci. Technol. 10, 1 - 14.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. (7th Rev. Ed.). National Academy. Press, Washington, D.C., USA. pp. 34 - 35.

Owens, F.N., Secrist, D.S., Hill, W.J. & Gill, D.R., 1998. Acidosis in cattle: a review. J. Anim. Sci. 76, 275 - 286.

Russel, J.B. & Wilson, D.B., 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci. 79, 1503 - 1509.

Sanson, D.W., Clanton, D.C., Rush, I.G., 1990. Intake and digestion of low-quality meadow hay by steers and performance of cows on native range when fed protein supplements containing various levels of corn. J. Anim. Sci. 68, 595 - 603.

Schwarz, F.J., Haffner, J. & Krichgessner, M., 1995. Supplementation of zero-grazed dairy cows with molassed sugar beet pulp, maize or cereal-rich concentrate. Anim. Feed Sci. Technol. 54, 237 - 248.

Simeone, A., Beretta, V., Rowe, J., Nolan, J., Elizalde, J.C. & Baldi, F., 2004. Rumen fermentation in Hereford steers grazing ryegrass and supplemented with whole or ground maize. In: Proceedings of the 25th Biennial Conference of the Australian Society of Animal Production, 4 - 8 July, University of Melbourne, Victoria, Australia. 25, 168 - 171.

Smith, L.W., Goering, H.K. & Gordon. C.H., 1972. Relationships of forage composition with rates of cell wall digestion and indigestibility of cell walls. J. Dairy Sci. 55, 1140.

Statistica 8.1., 2008. StatSoft Inc., USA.

Tomlin, D.C., Johnson, R.R. & Dehority. B.A., 1964. Relationship of lignification to in vitro cellulose digestibility of grasses and legumes. J. Anim. Sci. 23, 161.

Vallimont, J.E., Bargo, F., Cassidy, T.W., Luchini, N.D., Broderick, G.A. & Varga, G.A., 2004. Effects of replacing dietary starch with sucrose on ruminal fermentation and nitrogen metabolism in continuous culture. J. Dairy Sci. 87, 4221 - 4229.

Van Soest, P.J. & Robertson, J.B., 1985. Analysis of forages and fibrous feeds. A laboratory manual for animal science 613. Cornell University, Ithaca, New York, USA.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583 - 3587.

Voelker, J.A. & Allen, M.S., 2003. Pelleted beet pulp substituted for high-moisture corn: effect on rumianl fermentation, pH and microbial protein efficiency in lactating dairy cows. J Dairy Sci. 86 (11), 3562 - 3570.

Williams, V.J., Rottle, M.C., Moir, R.J. & Underwood, E.J., 1953. Ruminal flora studies in the sheep. IV. The influence of varying dietary levels of protein and starch upon digestibility, nitrogen retention, and the free microorganisms of the rumen. Aust. J. Biol. Sci. 6, 142.

Chapter 5

GENERAL CONCLUSION

The constant availability of high quality forages remains a problem in South Africa. The efficiency of forage utilization by ruminants is limited by several chemical and physical properties of forages, including a high fibre content and relative low energy content. The primary components of fibre are cellulose, hemicellulose, and lignin. Supplementing dairy cow diets with concentrates such as sugar, starch and pectin has the potential to improve animal performance by improving the degradability of forage feedstuffs.

Results from the current study suggested that the *in vitro methods* used were sufficient to indicate, not only that forages differ in terms of fermentability and digestibility, but also to show that different energy sources affect fermentation and digestion patterns of forages in different ways.

The first study indicated that molasses *per se* may have a negative effect on total forage fermentability (as determined by gas production), while citrus pulp may have a negative effect on the fermentability of certain forages, in this case oat hay and ryegrass. Maize meal did not affect forage fermentability as measured by total gas production. The study also suggested that citrus pulp and maize meal may increase the fermentation rate of oat hay, while maize may also increase the fermentation rate of kikuyu. The lag phase of wheat straw and kikuyu fermentation may be shortened by all the energy sources investigated, *viz.* maize meal, molasses and citrus pulp. It was concluded from the first study that forage fermentability is affected differently by different energy sources. These observations may have significant effects, in practice, on rumen health and milk production, and the data obtained can potentially be used as guide lines in feed formulations.

In the second study, it was shown that different energy sources had different effects on *in vitro* NDF digestibility (NDFD) of forages. In general, sucrose (after 24 and 72 hours of incubation) and pectin (72 hours) had no effect on NDFD of forage substrates. The supplementation of oat hay with starch and pectin (24 hours), and wheat straw with starch (24 and 72 hours), however, lowered NDFD when compared to the control treatment. It is hypothesized that micro-organisms fermented the easily fermentable energy sources first, before attacking forage NDF. The study suggested that forage NDF degradation values are not fixed, and may be altered by energy supplementation.

Understanding the interactions that exist between rumen pH, forages and NFC fractions used in dairy cow rations will help when formulating diets for lactating dairy cows. Knowledge of these concepts will aid in formulating diets that will ensure optimal NDFD, milk yield and milk composition, without causing ruminal health problems. Papers focusing on the topic of the effect of energy sources on forage digestibility and comparisons between *in vivo* and *in vitro* trials are limited and more research are needed in this regard.