THE EFFECTS OF SUPPLEMENTING ALTERNATIVE CARBOHYDRATE SOURCES ON PRODUCTION AND FIBRE DEGRADATION OF JERSEY COWS GRAZING PASTURE.

by
Lobke Steyn
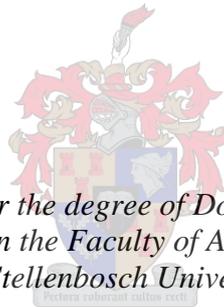
Dissertation presented for the degree of Doctor of Philosophy in Animal Science in the Faculty of AgriScience at Stellenbosch University

Promotor: Professor CW Cruywagen
Co-supervisor: Professor R Meeske

December 2017

DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualifications.

DATE: December 2017

ABSTRACT

Title	**:**	**The effects of supplementing alternative carbohydrate sources on production and fibre degradation of Jersey cows grazing pasture.**
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Supervisor	**:**	**Professor Christian W. Cruywagen**
Co-supervisor	**:**	**Professor Robin Meeske**
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Degree	**:**	**PhD in Animal Science**

Problems identified in the pasture-based dairy systems of the southern Cape of South Africa include lowered milk production during summer months, low milk solids during winter months, unsynchronised timing of pasture and concentrate feeding, lowered pasture degradability and pasture substitution. To counter act these problems and despite them, supplemental feeding is provided in the form of an energy rich concentrate, usually fed in the milking parlour. Historically, cereal grains form the largest part of the concentrate supplement and play an important role in determining the profitability of a dairy farm. The high starch content of cereal grains could have a limiting effect on microbial activity in the rumen due to lactic acid production, possibly resulting in low ruminal pH, which then impacts fibre degradation and has various negative production implications. Despite the problems associated with feeding starches it is still practised widely due to the high energy content, which promotes milk production. Other non-fibre carbohydrates, such as sugar and pectin (prevalent in various fruit wastes), have been shown to have a more positive effect on the rumen environment and are able to maintain production when substituted in total mixed ration systems. This study aimed to determine how effectively and to what degree alternative carbohydrate sources such as dried citrus pulp and dried apple pomace could be fed to Jersey cows grazing kikuyu pasture over-sown with ryegrass and what the possible production implications would be. The effect of dried citrus pulp and dried apple pomace on rumen metabolism and bacterial community dynamics was also investigated. The study consisted of three trials focused on the quality and application of dried citrus pulp and dried apple pomace. The first trial looked at the use of dried citrus pulp for cows grazing ryegrass pasture (*Lolium multiforum* var. Italicum, cv. Jeanne) and used 68 lactating Jersey cows (µ ± SD; 84.5 ± 43.8 days in milk, 20.4 ± 3.09 kg milk/day) allocated to one of four treatments in a complete randomised block design. Treatments were: No dried citrus pulp (NDCP)-0% replacement of ground maize, Low dried citrus pulp (LDCP)-33% replacement of ground maize, Medium dried citrus pulp (MDCP)-66%

replacement of ground maize and High dried citrus pulp (HDCP)-100% replacement of ground maize. An additional six ruminally cannulated, lactating Jersey cows were randomly allocated to the NDCP and HDCP treatments in a two period cross-over design. It was found that milk yield decreased between 2.1 and 3.2 kg/day when ground maize was substituted by dried citrus pulp. Milk fat content did not differ between treatments; however, treatment had a quadratic effect on milk protein and lactose content, with the LDCP and MDCP treatments having the highest content for both. No change in the diurnal ruminal pH curves and no differences in the rate and extent of pasture dry matter and neutral detergent fibre degradability between treatments were observed. It was concluded that replacing ground maize with dried citrus pulp was possible, but the large decrease in milk production was problematic. Furthermore, the lack of response of rumen metabolism and milk fat solids and the extremely low CP and high Ca content of DCP posed limitations on the use of dried citrus pulp as a replacer for ground maize. The composition of dried apple pomace is similar to dried citrus pulp, except that it possibly has a higher fibre, starch and protein content and is lower in Ca. Due to the unique composition of dried apple pomace and its proximity to the region, it was considered next. The second trial looked at the use of dried apple pomace for cows grazing kikuyu pasture. Seventy two lactating Jersey cows were blocked according to milk yield (mean $\pm$ SD; 16.1 ± 2.11 kg), days in milk (114 ± 46.2 d) and lactation number (3.8 ± 1.45) and randomly allocated to one of four treatments. Treatments were: 0% dried apple pomace inclusion (AP 0), 25% dried apple pomace inclusion (AP 25), 50% dried apple pomace inclusion (AP 50) and 75% dried apple pomace inclusion (AP 75). An additional eight ruminally cannulated, lactating cows were used and were subjected to a four period crossover design with a 14 day adaptation period between treatments. Although milk yield was not affected by the inclusion level of DAP, there was a linear decrease in 4% fat corrected milk (FCM) and fat yield as the level of dried apple pomace inclusion in the diet increased. Cows receiving the AP 0 concentrate supplement yielded 0.9 and 1.2 kg more 4% FCM than cows on both the AP 50 and AP 75 concentrate supplements (P <0.001), respectively. Treatment had no effect on milk composition, except for the lactose content, which was lower for cows receiving the AP 0 concentrate supplement (P <0.001). Mean rumen pH was lower for cows receiving the AP 75 concentrate supplement (P <0.001); however, treatment did not affect the volatile fatty acids (VFA) profile or pasture DM and NDF degradability. Here the use of dried apple pomace seemed viable; however, the lack of milk solids response and no improvement of the rumen environment were unfortunate. Due to the high fibre nature of kikuyu pasture the rumen environment is naturally under less stress when cows are grazing these summer pastures, as compared to winter pastures such as ryegrass that are more easily digestible and have lower physically effective NDF (peNDF) or rumen buffering

capacity. This trial was then essentially repeated on ryegrass pasture to determine whether the high fibre content of the dried apple pomace would be more effective in maintaining and possibly improving the rumen environment under more stressed conditions. In this third trial, 76 lactating Jersey cows were blocked according to milk yield (mean ± SE; 18.4 ± 0.01 kg), days in milk (97.2 ± 0.27 d) and lactation number (3.79 ± 0.04) and randomly allocated to one of four treatments. Treatments were: NDAP-0% dried apple pomace and 75% ground maize; LDAP-25% dried apple pomace and 50% ground maize; MDAP-50% dried apple pomace and 25% ground maize; HDAP-75% dried apple pomace and 0% ground maize. Additionally, four ruminally cannulated cows were used to monitor treatment effect on rumen activity and health. Milk yield and 4% FCM yield were lower for cows in treatment HDAP than for cows in treatments NDAP and LDAP, differences ranging between 1.7 and 2.3 kg 4% FCM/day. The milk protein yield remained unchanged between treatments, whereas milk protein content was lowest for cows in treatments NDAP and MDAP, showing a cubic trend (P = 0.005). Treatment had no effect on rumen metabolism parameters. In this trial it was determined that dried apple pomace could sustain milk production on ryegrass pasture; however, milk solids could possibly be negatively impacted.

In addition to the production and rumen metabolism studies, a ruminal bacterial community dynamics study was also undertaken. Rumen fluid samples were collected for further study from cannulated cows in the second and third trials. It was interesting to note that the composition of the bacterial community was affected by a change in diet, even though that was not always reflected in the rumen metabolism (pH, VFA concentration and pasture degradation). The detailed description of the ruminal bacterial community will be of great value for future research regarding the nutrition of dairy cows grazing pasture and was the first of its kind.

In conclusion, this research has provided insight into the use of fruit waste as a feed for dairy cows in pasture-based systems in a South African context. There are various limitations regarding the application thereof, but both dried citrus pulp and dried apple pomace are feed sources with potential as a ruminant feed and should not be over-looked by farmers and feed processors alike.

UITTREKSEL

Titel	**:**	**Die effek van die gebruik van alternatiewe koolhidraatbronne vir aanvulling van Jerseykoeie op produksie en weidingsverteerbaarheid.**
Kandidaat	**:**	**Lobke Steyn**
Studieleier	**:**	**Professor Christian W. Cruywagen**
Medestudieleier	**:**	**Professor Robin Meeske**
Instansie	**:**	**Departement van Veekundige Wetenskappe, Universiteit van Stellenbosch**
Graad	**:**	**PhD in Veekunde**

Probleme wat in die weidingsgebaseerde suiwelstelsels van die Suid-Kaap van Suid-Afrika voorkom, sluit in die verminderde melkproduksie gedurende die somermaande, lae melkstowwe gedurende die wintermaande, gesinkroniseerde tydsberekening van weiding en kragvoervoeding, verlaagde weiding afbreekbaarheid en weiding vervanging. Om nie net hierdie probleme aan te spreek nie, maar ook ongeag daarvan, word aanvullende voeding verskaf in die vorm van 'n energieryke kragvoer wat gewoonlik in die melkstal gevoer word. Histories maak grane die grootste deel van die kragvoeraanvulling uit en speel 'n belangrike rol in die bepaling van die winsgewendheid van 'n melkplaas. Die hoë styselinhoud van grane het 'n beperkende effek op mikrobiese aktiwiteit in die rumen as gevolg van melksuurproduksie, wat moontlik tot lae rumen pH lei, wat dan veselverteerbaarheid beïnvloed en verskeie negatiewe produksie-implikasies het. Ten spyte van die probleme wat verband hou met die voeding van stysels, word dit steeds wyd beoefen weens die hoë energie-inhoud wat melkproduksie bevorder. Ander nie-vesel koolhidrate, soos suiker en pektien (voorkomend by 'n verskeidenheid van vrugte afval), het in vorige navorsing getoon dat dit 'n positiewer effek op die rumen-omgewing het en in staat is om produksie te handhaaf wanneer dit in totale gemengde rantsoene vervang word. Hierdie studie was daarop gemik om te bepaal hoe doeltreffend en tot watter mate alternatiewe koolhidraatbronne (soos gedroogte sitruspulp en gedroogde appelpulp) aan Jerseykoeie op kikoejoeweiding, oorgesaai met raaigras, gevoer kan word. Die invloed van gedroogde sitruspulp en gedroogde appelpulp op rumenmetabolisme en bakteriese gemeenskapsamestelling is ook ondersoek. Die studie het bestaan uit drie proewe wat fokus op die gehalte en gebruik van gedroogde sitrus- en appelpulp. Die eerste proef het gekonsentreer op die gebruik van gedroogde sitruspulp vir koeie wat op raaigras wei. Hiervoor is 68 lakterende Jerseykoeie

(μ ± SD, 84,5 ± 43,8 dae in melk, 20,4 ± 3,09 kg/dag) in vier behandelings gebruik waar mielies in die kragvoeraanvulling inkrementeel met gedroogte sitruspulp vervang is. Behandelings was: Geen gedroogde sitruspulp (NDCP) - 0% vervanging, Lae gedroogde sitruspulp (LDCP) - 33% vervanging, Medium gedroogde sitruspulp (MDCP) - 66% vervanging en Hoë gedroogte sitruspulp (HDCP) - 100% vervanging . 'n Bykomende ses gekannuleerde Jerseykoeie is ewekansig aan die NDCP en HDCP behandelings toegeken. Daar is bevind dat melkopbrengs tussen 2.1 en 3.2 kg/dag afgeneem het toe mielies met gedroogde sitruspulp vervang is. Melkvet-inhoud het nie tussen behandelings verskil nie. Behandeling het egter 'n kwadratiese effek op melkproteïen en laktose-inhoud gehad, met die LDCP en MDCP behandelings wat die hoogste inhoud gehad het. Geen verskille is tussen behandelings vir diurnale rumen pH en weidingsverteerbaarheid waargeneem nie. Daar is bevind dat die vervanging van mielies met gedroogde sitruspulp haalbaar was, maar dat die groot afname in melkproduksie problematies was. Verder het die gebrek aan reaksie ten opsigte van rumenmetabolisme en melkvet, asook die lae RP waarde en hoë Ca waarde beperkings op die gebruik van gedroogde sitruspulp as 'n vervanger vir mielies geplaas. Die samestelling van gedroogde appelpulp is soortgelyk aan dié van gedroogde sitruspulp, behalwe dat dit moontlik 'n hoër vesel-, stysel- en proteïeninhoud het en laer in Ca is. As gevolg van die unieke samestelling van appelpulp, asook die voorkoms daarvan binne die omliggende streek, is dit in die volgende proewe evalueer. Die tweede proef het gefokus op die gebruik van gedroogde appelpulp vir koeie wat op kikoejoeweiding aangehou word. Twee-en-sewentig lakterende Jerseykoeie is volgens melkopbrengs, dae in melk en laktasienommer geblok en ewekansig aan een van vier behandelings toegeken waar mielies in die kragvoeraanvuling inkrementeel met gedroogte appelpulp vervang is. Behandelings was: 0% gedroogde appelpulp insluiting (AP 0), 25% gedroogde appelpulp insluiting (AP 25), 50% gedroogde appelpulp insluiting (AP 50) en 75% gedroogde appelpulp insluiting (AP 75). 'n Bykomende agt rumengekannuleerde koeie is ingesluit. Die behandelings het geen invloed op melkopbrengs gehad nie. Daar was egter 'n lineêre afname in 4% FCM en vet-opbrengs met 'n toename in gedroogde appelpulp insluiting in die kragvoeraanvulling. Koeie wat die AP 0 kragvoer gekry het, het 0.9 en 1.2 kg meer 4% FCM per koei opgelewer as die koeie wat AP 50 en AP 75 kragvoeraanvullings ontvang het (P <0.001). Die behandelings het geen invloed op melksamestelling gehad nie, behalwe vir die laktose-inhoud wat laer was vir koeie wat die AP 0 kragvoer (P <0.001) ontvang het. Gemiddelde rumen pH was laer vir koeie wat die AP 75 kragvoer (P <0.001) ontvang het. Die behandelings het egter nie die vlugtige vetsuurprofiel of weidingverteerbaarheid beïnvloed nie. Hier was die gebruik van gedroogde appelpulp moontlik, maar die gebrek aan 'n reaksie op melksamestelling en die verbetering van die rumenomgewing was egter onverwags. As gevolg van die relatief hoë

veselinhoud van kikoejoeweiding, is die rumenomgewing onder minder stres wanneer koeie hierdie tipe somerweiding bewei in vergelyking met winterweiding, soos raaigras, wat makliker verteerbaar is. Hierdie proef is herhaal op raaigrasweiding om vas te stel of die hoë veselinhoud van die gedroogde appelpulp meer doeltreffend sou wees om die rumenomgewing onder meer stremmende omstandighede te verbeter. In die derde proef is 76 lakterende Jerseykoeie volgens melkopbrengs, dae in melk en laktasienommer geblok en ewekansig aan een van vier behandelings toegeken. Behandelings was: NDAP - 0% gedroogde appelpulp en 75% mielies, LDAP - 25% gedroogde appelpulp en 50% mielies, MDAP - 50% gedroogde appelpulp en 25% mielies en HDAP - 75% gedroogde appelpulp en 0% mielies. Vier rumengekannuleerde koeie is ook ingesluit om die behandelingseffek op rumenaktiwiteit en -gesondheid te monitor. Die 4% FCM was tussen 1.7 en 2.3 kg/dag laer vir koeie in behandeling HDAP as vir koeie in behandelings NDAP en LDAP. Die melkproteïeneopbrengs het onveranderd gebly tussen behandelings, terwyl die melkproteïeninhoud die laagste was vir koeie in behandelings NDAP en MDAP. Laasgenoemde het 'n kubieke tendens getoon (P = 0.005). Die behandelings het geen invloed op rumenmetabolisme parameters gehad nie. In hierdie proef is vasgestel dat gedroogde appelpulp melkproduksie op raaigrasweiding kan onderhou, hoewel melkvastestowwe moontlik negatief beïnvloed kan word.

Afgesien van die melkproduksie en rumenmetabolisme studies is 'n rumen bakteriese samestellingstudie ook onderneem. Rumenvloeistofmonsters is in die tweede en derde proewe van gekannuleerde koeie versamel om die populasiesamestelling na te gaan. Dit was opvallend dat die samestelling van die bakteriese gemeenskap beïnvloed is deur 'n verandering in dieet, hoewel dit nie altyd in die rumenmetabolisme (pH, vlugtige vetsuurkonsentrasie en weidingsverteerbaarheid) weerspieël is nie. Hierdie was die eerste studies van sy soort en die gedetailleerde beskrywing van die rumen bakteriese gemeenskap sal van groot waarde wees vir toekomstige navorsing aangaande die voeding van melkkoeie op weiding.

Ten slotte het hierdie navorsing insig verskaf in die gebruik van vrugteafval as byvoeding vir melkkoeie in weiding-gebaseerde stelsels in 'n Suid-Afrikaanse konteks. Daar is verskeie beperkings ten opsigte van die toepassing daarvan, maar beide gedroogde sitruspulp en gedroogde appelpulp is voerbronne met potensiaal in herkouervoeding en moet nie deur boere en voermaatskappye oorgesien word nie.

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But as for me, it is good to be near God.

I have made the Sovereign Lord my refuge;

I will tell of all Your deeds.

Psalm 73: 28

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADL	Acid detergent lignin
BCS	Body condition score
BCVFA	Branched chain volatile fatty acids
BW	Body weight
CP	Crude protein
DAP	Dried apple pomace
DCP	Dried citrus pulp
DIM	Days in milk
DM	Dry matter
DMD	Dry matter degradability
DMI	Dry matter intake
EE	Ether extract
FCM	Fat corrected milk
GE	Gross energy
iNDF	Indigestible neutral detergent fibre
IVOMD	In vitro organic matter digestibility
ME	Metabolisable energy
MJ	Mega joules
MP	Microbial protein
MUN	Milk urea nitrogen
NDF	Neutral detergent fibre
NDFD	Neutral detergent fibre degradability
NDSC	Neutral detergent soluble carbohydrate
NDSF	Neutral detergent soluble fibre
NE_L	Net energy for lactation
NFC	Non-neutral detergent fibre carbohydrates
NFFS	Non-forage fibre source
NH_3-N	Ammonia nitrogen
NSC	Non-structural carbohydrates
OM	Organic matter
peNDF	Physically effective neutral detergent fibre
RDP	Rumen degradable protein
RPM	Rising plate meter
RUP	Rumen undegradable protein
SCC	Somatic cell count
TMR	Total mixed ration
VFA	Volatile fatty acids
WSC	Water soluble carbohydrates

Chapter 1: Introduction

1.1 Background

Pasture-based milk production systems in the southern Cape area of South Africa are predominantly based on kikuyu (*Pennisetum clandestinum*) pasture, over-sown with annual ryegrass (*Lolium multiforum*), where cows are supplemented with a high starch concentrate as an energy supplement in the milking parlour to overcome the energy limitations of pasture. Maize is currently the most common carbohydrate component used to overcome the nutritional limitations of pasture; however, the high starch content of maize has a limiting effect on microbial activity in the rumen due to lactate production, often resulting in low ruminal pH, at high levels of inclusion. Low ruminal pH reduces fibre degradation and has various negative production implications. Despite the problems associated with feeding starches it is still practised widely because of the high content of ME which is rapidly available to the cows. As such the inclusion of starches into a lactating cow ration is always accompanied by an increase in milk production, which is due to the higher energy density of the diet and not necessarily due to improved utilisation of feeds and pasture. Other carbohydrate components, such as sugar and pectin, have been shown to have a positive effect on the rumen environment and maintain production when used in TMR systems. Considering alternative carbohydrate sources for Jersey cows on pasture will provide a clearer understanding of the different carbohydrate components and the potential for increased production (yield, composition, cow health) or maintained production at lower costs. Furthermore, diets differing in the primary energy source are expected to result in a change in the bacterial community dynamics of the rumen. Up to date very little is known regarding the bacterial community of cows grazing pasture, much less, of cows receiving varying levels of sugar and starch. The information derived from this study will provide insight into the *in vivo* effects of alternative carbohydrate sources supplemented to cows on summer and winter pasture. Information of the bacterial community dynamics of the rumen under various different feeding strategies will also prove a valuable resource for the future comprehension of energy source effects. It may also be advantageous to the animal feed industry as alternative sources could be recommended for inclusion into concentrate diets with more confidence in the potential production effects at lower costs. Stimulating the use of by-products on the basis of scientific reasoning will benefit South Africa immensely as it would allow for the effective utilisation of sources that are regarded as waste and would provide opportunities for a cleaner production cycle with less wastage. In 2015-2016 South Africa

produced 1.7 million tons of oranges and 920 000 tons of apples, of which 25% and 30% were purchased for further processing, respectively (DAFF, 2017). Of the 400 000 ton oranges and 276 000 ton apples purchased for processing in 2015-2016 (including juicing, tinning, making jam etc.) it is not known exactly how much waste and potential animal feed was generated. If a third of the apples purchased for processing were pressed for juice (no figures available for this) and all the residues were to be collected and dried it would have yielded an average of 3 825 ton of dried apple pomace. In comparison to maize this amount is small; however, it could bring great financial relief for dairy farmers in areas that have easy access to these products. When orange residue is also considered along with all other fruit and vegetable waste, there is a potential animal feed source that is not currently being managed or supervised to any great extent. The unique climate and production systems and areas in South Africa necessitate a deeper understanding of the application of these by-products in a South African context. Furthermore, the ever increasing human population, estimated at 9 billion by 2050, necessitates more effective utilisation of waste products, limiting animal use of feeds that could potentially be used as human food.

1.2 Problem statement/Research questions

Problems identified in the southern Cape of South Africa include lowered milk production during summer months, low milk solids during winter months, unsynchronised timing of pasture and concentrate feeding, lowered pasture degradability and pasture substitution when concentrate is supplemented. The aim of this study was thus to determine how effectively alternative carbohydrate sources such as dried citrus pulp and dried apple pomace can be fed to Jersey cows grazing kikuyu pasture over-sown with ryegrass and what the possible production implications would be. The effect of dried citrus pulp and dried apple pomace on rumen metabolism and bacterial community dynamics was also determined.

1.3 Thesis layout

The language and style used in this dissertation are in accordance with the requirements of the *South African Journal of Animal Science*. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has been unavoidable. Furthermore, various chapters have been published as original research articles in peer reviewed journals or are in the process of being reviewed and have thus been included as submitted or published.

1.4 Research outputs

1.4.1 Publications

1.4.1.1 Popular

- Steyn, L., Meeske, R., Cruywagen, C.W., 2014. The effect of substituting maize grain with citrus pulp on the production of Jersey cows grazing ryegrass pasture. Information day: Milk production from planted pastures. Outeniqua Research Farm, Western Cape Department of Agriculture.
- Steyn, L., 2015. *Alternatiewe koolhidraatbronne vir koeie op weiding*. Afgriland, 1 January 2015, 26.
- Coetsee, J., 2015. *Kragvoer: Vervang mielies met afvalprodukte*. *Landbou Weekblad*, 12 July 2015, No. 1908.
- Steyn, L., Meeske, R., Cruywagen, C.W., 2015. The effect of substituting maize grain with citrus pulp on production of Jersey cows grazing ryegrass pasture. AgriProbe, Elsenburg Journal, 12 (1), 53-54.
- Steyn, L., 2015. The maize alternative. The Dairy Mail, November, 82-87.
- Steyn, L. 2017. *Gedroogte appelpulp: 'n alternatief vir mielies*? Afgriland 61 (3): 38-39.

1.4.1.2 Poster

- Steyn, L., Meeske, R., Cruywagen, C.W., 2015. Rumen response of Jersey cows grazing ryegrass pasture supplemented with a high maize or high citrus pulp concentrate. 48th SASAS congress, South Africa.
- Steyn, L., Meeske, R., Cruywagen, C.W., 2017. The effect of replacing maize with dried apple pomace on rumen parameters for cows grazing kikuyu pasture. 50th SASAS congress, South Africa.

1.4.1.3 Scientific

- Steyn, L., Meeske, R., Cruywagen, C.W., 2017. Replacing maize grain with dried citrus pulp in a concentrate feed for Jersey cows grazing ryegrass pasture. South African Journal of Animal Science 47: 553-564. (doi: org/10.4314/sajas.v47i4.14)
- Steyn, L., Meeske, R., Cruywagen, C.W., 2017. The effect of replacing maize with dried apple pomace in the concentrate on performance of Jersey cows grazing kikuyu pasture. Submitted for review to Animal Feed Science and Technology on 13 July 2017.
- Steyn, L., Meeske, R., Cruywagen, C.W., 2017. The effect of dried apple pomace as a replacer for maize in the concentrate for Jersey cows grazing ryegrass pasture on production and rumen metabolism. Animal Feed Science and Technology 243: 264-273. (doi: org/10.1016/j.anifeedsci.2017.10.011)

1.4.2 Platform presentations

1.4.2.1 Scientific congress

- Steyn, L., Meeske, R., Cruywagen, C.W., 2014. The effect of substituting maize grain with citrus pulp on the production of Jersey Cows grazing ryegrass pasture. 47th SASAS congress, South Africa.
- Steyn, L., Meeske, R., Cruywagen, C.W., 2016. The effect of increasing sugar and pectin content in the concentrate on milk production and composition of dairy cows grazing kikuyu-ryegrass pasture in summer. 49th SASAS congress, South Africa.
- Steyn, L., Meeske, R., Cruywagen, C.W., 2017. The effect of replacing maize with dried apple pomace on production of cows grazing ryegrass pasture. 50th SASAS congress, South Africa.

1.4.2.2 Formal presentation by invitation

- Steyn, L., Meeske, R., Cruywagen, C.W., 2014. The effect of substituting maize grain with citrus pulp on the production of Jersey cows grazing ryegrass pasture. Information day: Milk production from planted pastures. Information day, Outeniqua Research Farm, Western Cape Department of Agriculture.
- Steyn, L., Meeske, R., Cruywagen, C.W., 2016. The effect of substituting maize grain with apple pomace in a concentrate on the production of Jersey cows grazing kikuyu-ryegrass pasture in summer. Information day: Milk production from planted pastures. Outeniqua Research Farm, Western Cape Department of Agriculture.
- Steyn, L., Meeske, R., Cruywagen, C.W., 2017. The potential use of dried apple pomace as the main energy source for Jersey cows grazing ryegrass pasture. Information day: Milk production from planted pastures. Outeniqua Research Farm, Western Cape Department of Agriculture.

1.4.2.3 Radio

- Steyn, L., 2014. *Die gebruik van alternatiewe koolidraat bronne vir koeie op weiding*. Radio Elsenburg, RSG.
- Steyn, L., 2017. *Die gebruik van gedroogte appel-pulp as 'n alternatief vir mielies*. Radio Elsenburg, RSG

Chapter 2: Literature review

2.1   Introduction

General practice for pasture-based dairy systems includes the allocation of pasture and supplementation of a high maize/high starch concentrate feed. This system ensures high production of milk, but it is not biologically efficient. The current environmental stresses and economic pressures require that such highly intensive systems operate at a more efficient level, with minimum input for maximum output. High output is essential, as there are certain requirements that are placed on the agricultural sector by increasing population numbers. Improving the efficiency of milk production lies in increasing the degradability of pasture, the cheapest and main feed component in pasture-based dairy systems. Understanding the effects of the various energy sources for cows on pasture will provide the gateway for creating more efficient systems.

2.2   Carbohydrates in nutrition

Carbohydrates play a crucial role in the supply of energy to dairy cows and are essential for yielding high levels of milk production (Allen & Knowlton, 1995) and up to 70% of a dairy ration can be made up of carbohydrates (NRC, 2001). Carbohydrates can be divided into two broad categories: non-fibre carbohydrates (NFC) also referred to as non-neutral detergent fibre carbohydrates or neutral detergent soluble carbohydrates (NDSC) and neutral detergent fibre (NDF), Figure 2-1 (Englyst & Hudson, 1996; Ariza *et al.*, 2001; McDonald *et al.*, 2010; Hall, 2011). The NFC component can be further subdivided into non-structural carbohydrates (NSC) and neutral detergent soluble fibre (NDSF). The NSC component is divided into water soluble carbohydrates (WSC), which includes organic acids, mono-, and oligosaccharides, otherwise known as sugar (e.g. glucose, sucrose and lactose) and water insoluble carbohydrates that include homoglycan polysaccharides such as starch and inulin (Allen & Knowlton, 1995). The NDSF component includes the readily fermentable carbohydrates which form part of the cell wall and as such cannot be digested by mammalian enzymes and include homoglycan polysaccharides (fructans) and heteroglycan polysaccharides (pectic substances; e.g. β-glucans and galactans) (Ariza *et al.*, 2001; McDonald *et al.*, 2010). The terms NSC and NFC are often used interchangeably but from the above classification it is clear that they do not refer to the same components. To clarify, the term NSC includes organic acids, mono- and oligosaccharide as well as homoglycan polysaccharides, whereas the term NFC includes all components of

NSC as well as heteroglycan polysaccharides (Hall, 2011).
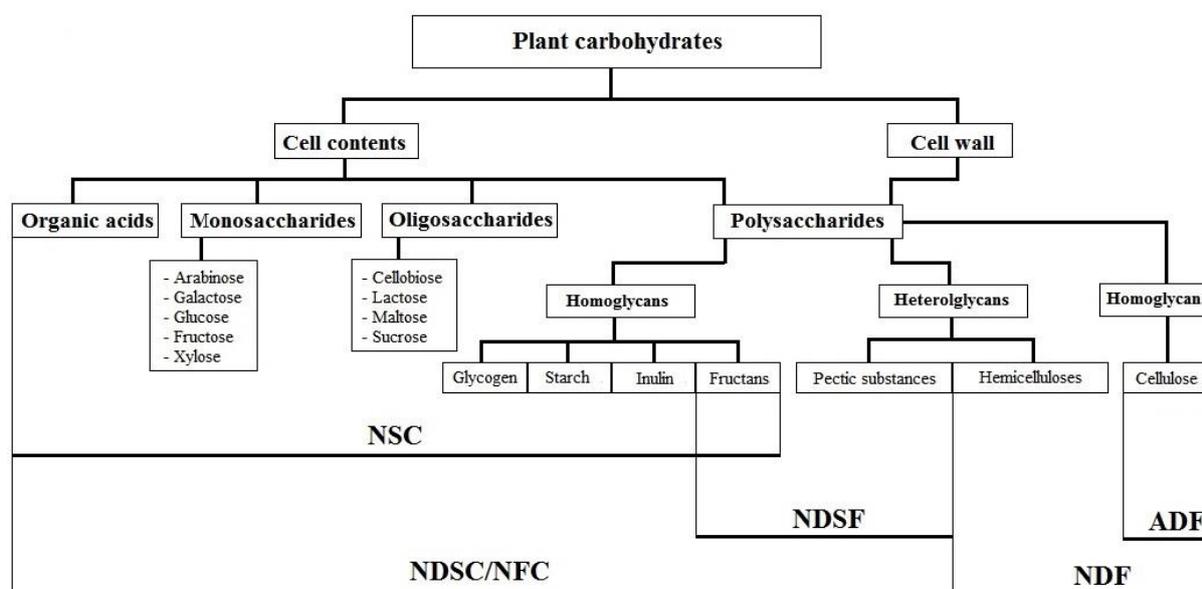
Figure 2-1 Schematic representation of the classification of plant carbohydrates. NDSF-Neutral detergent soluble fibre, NDSC - Neutral detergent soluble carbohydrate, NSC–Non-structural carbohydrates, NFC–Non-neutral detergent fibre carbohydrates, NDF–Neutral detergent fibre, ADF–Acid detergent fibre.

The NFC component is the primary source of energy for rumen micro-organisms. The NDF fraction is comprised of cellulose and hemicellulose and these are considered to be partially indigestible or slowly digestible in the rumen. The NDF fraction also plays a role in providing energy to the micro-organisms but this is only secondary to its role in the maintenance of rumen and gut health (Allen, 1997; Zebeli *et al.*, 2012). The NFC component is complex as it contains structural and non-structural carbohydrates, as well as fibrous and non-fibrous carbohydrates. The NFC component is often considered a single entity when diets are formulated for ruminants and does not consider the diverse nutritional characteristics of the various components (Ariza *et al.*, 2001). Through extensive research on the different components it is clear that treating all the components of NFC and NSC of different feeds as a uniform entity is not warranted and the same applies to concentrate supplementation on pasture-based systems.

2.2.1 Pectin

Although pectin forms part of the plant cell wall it is not covalently linked to the lignified portions of the cell wall and can therefore be digested by rumen micro-organisms (Van Soest *et al.*, 1991; NRC, 2001). As such pectin is not classified under the NDF component but rather the NFC component and more specifically forms part of the NDSF fraction, which means that

it is essentially a soluble fibre (Leiva *et al.*, 2000; Hall & Herejk, 2001). Pectin is almost completely fermented in the rumen, up to 90-100% (Nocek & Tamminga, 1991; Titgemeyer *et al.*, 1992; NRC, 2001) and is fermented more rapidly than hemicellulose and cellulose (Marounek *et al.*, 1985). Pectin is found in a wide range of dairy feeds, generally in low concentrations (20-30 g/kg DM; Allen, 2001). There are however a number of feeds that contain a considerable amount of pectin, these include citrus pulp (~ 150 g/kg DM), beet pulp (150-250 g/kg DM; Marounek *et al.*, 1985) and lucerne (30-100 g/kg DM; Van Soest *et al.*, 1991; Allen, 2001).

2.2.2 Starch

Starch is a storage polysaccharide of plants and comprises up to 70-80% of most cereal grains (Rooney & Pflugfelder, 1986). Starch is composed of two major molecules, namely amylopectin and amylose (Rooney & Pflugfelder, 1986). Amylopectin is a branched polymer with linear chains of α-(1→4) and α-(1→6) linkages and comprises 70-80% of most cereal starches. Amylose is a linear polymer of α-(1→4) linked glucose units and comprises 20-30% of cereal starches. Starch is highly fermentable in the rumen, ranging from digestibility of 40-90% (NRC, 2001), depending on the structure (amylopectin/amylose ratio), plant source and physical form or processing (Russell *et al.*, 1992; Allen, 2001; Niwińska, 2012). Starch can be further degraded by enzymes in the small intestines (Niwińska, 2012).

2.2.3 Sugar

Sugar also falls under the carbohydrate fraction of NSC but differ from starches as they are classified as WSC and include mono-, di- and oligosaccharides, with monomers linked through α-(1→4) linkages (Holtshausen, 2004; Oba, 2011; Niwińska, 2012). Sugar is completely degraded in the rumen (Sniffen *et al.*, 1992) and the Cornell Net Carbohydrate and Protein System (CNCPS) assumes a fermentation rate of up to 300%/hour (Oba, 2011). However, a small proportion (< 5%) of lactose, a glucose and galactose polymer, might escape rumen fermentation and become available for enzymatic digestion in the duodenum (Weisbjerg *et al.*, 1998). Sugar has also been shown to increase the production of butyrate in the rumen (Ribeiro *et al.*, 2005; Oba, 2011; Hall, 2011) and it is speculated that milk fat % increases as a result of this (Broderick *et al.*, 2008).

2.3 Pasture for dairy cows

2.3.1 Pasture degradability

The low metabolisable energy (ME) content of pasture is the primary factor limiting milk production from pasture (Kolver & Muller, 1998; Jacobs, 2014). As such it is common practise to feed an energy based supplement to cows on pasture so as to achieve a target milk production and milk composition (Jacobs, 2014). The high crude protein (CP) content of ryegrass (214-298 g/kg DM; Joubert, 2012; Van Wyngaard *et al.*, 2015) and kikuyu (201-229 g/kg DM; Van der Colf, 2011; Cawood, 2016) pasture can result in low ruminal N capture (Gehman *et al.*, 2006) and limits milk production (Carruthers & Neil, 1997). To ensure utilisation of NH_3-N by rumen micro-organisms it is important to provide cows with a readily fermentable carbohydrate source that will be degraded in synchronisation with the CP of pasture (Carruthers & Neil, 1997; Trevaskis *et al.*, 2004). Further compounding the lack of effective utilisation of pasture is the sensitivity of proteolytic, pectinolytic and cellulolytic bacteria to ruminal pH. When supplemental concentrates high in starch are fed to cows on pasture energy requirements are met but often at the expense of ruminal pH. Decreased activity of micro-organisms will result in a lower rate of degradation of pasture, ultimately lowering dry matter intake (DMI) and milk production (Berzaghi *et al.*, 1996).

2.3.2 Nitrogen use efficiency on pasture

Only 5-15% of all N applied to agricultural land is ultimately transformed into human food (Erisman *et al.*, 2011). Nitrogen management is of particular concern in pasture-based dairy systems where high levels of fertiliser are applied to pasture so as to ensure high pasture production for overall farm productivity (Higgs *et al.*, 2013). This problem is compounded by the fact that high quality pastures, such as perennial ryegrass (*Lolium perenne*), generally contain higher levels of CP (> 230 g/kg DM) than is required by a small breed dairy cow (120-160 g/kg DM; NRC, 2001; Dewhurst, 2006). Excessive CP intake leads to high levels of urinary N excretion, which ultimately leads to significant environmental consequences, especially impacting water quality in aquifers, rivers and lakes (Mulligan *et al.*, 2004; Dewhurst, 2006; Erisman *et al.*, 2011; Higgs *et al.*, 2013). High CP intake also results in high ruminal N, blood urea nitrogen and milk urea nitrogen (MUN) levels (Gehman *et al.*, 2006). Nitrogen use efficiency depends on the amount of N fed, the type and amount of concentrate fed, as well as the productive output of N from the cow (Dewhurst, 2006; Calsamiglia *et al.*, 2010; Higgs *et al.*, 2013). Any N that is not secreted in milk or accreted into tissue will be

excreted in urine or faeces (Lapierre & Lobley, 2001). Typically N use efficiency is low in ruminants, ranging from 15-40%, averaging around 25% and is further compromised by feeding diets that support high levels of cow productivity (Calsamiglia *et al.*, 2010). Similarly N input and milk production is strongly correlated, where high N inputs result in high milk production (Gourley *et al.*, 2011). In a study by Gourley *et al.* (2011), the surplus amount of N (g) per litre of milk produced was used as a measure for N utilisation efficiency and ranged from 12.2-14.5 g N/L milk in Australian dairies. As the content of N in the diet is increased the efficiency of utilisation is decreased and the amount of urinary N is increased. This curvilinear relationship is described by Castillo *et al.* (2000) as: Urine N (g/d) = 30.4 ($e^{0.0036 * N \text{ intake (g/d)}}$).

High stocking rates on high quality pasture further compounds the problem of urinary N excretion on pasture (Dewhurst, 2006). Additionally, the application of N fertiliser also plays a role in N use efficiency, where increased use of N fertiliser decreased the conversion of pasture N into milk N (Peyraud & Astigarraga, 1998). Genetic breeding alone provides little scope for improving N utilisation. The importance of producing pasture that is highly digestible is of greater consequence than increasing the WSC content, thereby decreasing the CP content (Marais *et al.*, 2003; Dewhurst, 2006; Jacobs, 2014). It is also unlikely that N fertilisation rates will be lowered below the environmental restrictions as this will lead to lowered pasture yields (Jacobs, 2014). Instead the greatest opportunity to ameliorate N use efficiency lies in the manipulation of the dairy cow concentrate fed and improving ration balancing, particularly with regards to the CP balance as CP balance is the single most important factor determining the N use efficiency (Hall & Herejk, 2001; Erisman *et al.*, 2011; Higgs *et al.*, 2013; Jacobs, 2014). The use of high protein supplements will exacerbate the problem of low N efficiency (Dewhurst, 2006; Higgs *et al.*, 2013). Therefore, the main goal with regards to N use efficiency is to find the balance between an environmentally sustainable dairy system and an economically sustainable dairy system (Ryan *et al.*, 2011; Jacobs, 2014).

2.4   Non-fibre carbohydrates in dairy concentrates

2.4.1 Source of NFC

2.4.1.1   Forages

As forages make up the majority of a dairy cow diet it is important to consider the NFC profile of specific sources, in this instance ryegrass and kikuyu pasture. As a note, the term WSC is used synonymously with NFC for pasture. Temperate species (e.g. annual ryegrass; *Lolium multiflorum*) tend to have a higher WSC compared to tropical and sub-tropical grasses (e.g. kikuyu; *Pennisetum clandestinum*) (Shao *et al.*, 2005). Forages vary widely in WSC content (20-57 g/kg DM; Fulkerson *et al.*, 2007); however, it can be as high as 100-150 g/kg DM in forages that have been specifically bred for higher WSC (Lee *et al.*, 2002). As the WSC content of pasture increases, referring to the NFC component, the starch content also increases and the NDF content decreases (Oba, 2011), yielding a more degradable pasture. The WSC content of pasture is highest during the late afternoon as WSC is a by-product of photosynthesis and accumulate throughout the day (Oba, 2011). During the night time WSC are used by the plant for respiration, as such the WSC content of pasture is the lowest during the early morning (Trevaskis *et al.*, 2001; Oba, 2011). The pectin content of pasture ranges between 30-40 g/kg DM (Marounek & Dušková, 1999). Ryegrass pasture has a minimum CP content of 250 g/kg DM, of which at least 90 g/kg DM is available as soluble protein (McCormick *et al.*, 2001). The soluble CP is readily deaminated to NH_3, which is mostly absorbed into the bloodstream and excreted as urea, when an adequate supply of NFC is not available (Marais *et al.*, 2003). This asynchrony in the supply of nutrients, which negatively affects the microbial activity, is prevalent with high CP ryegrasses (McCormick *et al.*, 2001; Fulkerson *et al.*, 2007). In kikuyu the asynchrony of nutrient supply is aggravated by the high NDF, subsequently lowering the digestibility of the pasture (Marais, 2001). Kikuyu pasture has a CP content ranging between 129-228 g/kg DM and a NDF content ranging between 552-637 g/kg DM (Garcia *et al.*, 2014).

2.4.1.2   Dried citrus pulp

Citrus pulp is the residue which remains after juice is extracted and is comprised of peel, pulp and seeds in varying concentrations (Bampidis & Robinson, 2006). Each of the different components of citrus pulp have a unique nutritional profile, therefore the quality of citrus pulp depends on the composition of different components. Typically citrus pulp, after juice extraction, is made up of 600-650 g/kg DM peel, 300-350 g/kg DM pulp and 0-100 g/kg DM

seeds (Pascual & Carmona, 1980). Citrus pulp is then further subjected to a drying process, which includes shedding, liming, pressing and drying, eventually yielding a citrus pulp with around 920 g/kg DM (Bampidis & Robinson, 2006). In general, dried citrus pulp (DCP) is high in sugar and pectin and low in starch, compared to maize, which is a more conventional energy source, Table 2-1. One of the biggest limitations of DCP for the animal feed sector is the possibly low and highly varying CP content (Miller-Webster & Hoover, 1998; NRC, 2001). The use of DCP in dairy rations is common place for total mixed ration (TMR) systems, where it acts as a flavour enhancer, often promoting feed intake due to the high sugar content (Bampidis & Robinson, 2006; Penner & Oba, 2009). Even though it is a well-known product little attention is given to the NFC profile. The DCP is unique due to the high pectin content (Bampidis & Robinson, 2006) and the high NDF content (Miller-Webster & Hoover, 1998; NRC, 2001). The unique nutritional composition of DCP makes it a viable feed option for ruminants.

2.4.1.3 Dried apple pomace

Dried apple pomace (DAP) is the by-product that results from the pressing of apples for juice (Kennedy *et al.*, 1999) and then drying the pomace. Dried apple pomace is high in pectin (Hindrichsen *et al.*, 2004; Mirzaei-Aghsaghali & Maheri-Sis, 2008) and sugar (Miller-Webster & Hoover, 1998), Table 2-1. The NDF, ADF and ADL content of DAP is also high (Edwards & Parker, 1995; NRC, 2001; Mirzaei-Aghsaghali *et al.*, 2011). As is the case with DCP, the chemical composition of DAP varies widely, depending on the processing methods applied as well as the specific apple variety and how it was managed post-harvest (Kennedy *et al.*, 1999; Abdollahzadeh *et al.*, 2010). Apple pomace (dried or wet ensiled) has been used with success in TMR diets fed to lactating dairy cows (Edwards & Parker, 1995); however, little information is available on AP fed to cows grazing pasture as the main roughage source.

2.4.1.4 Molasses

Both cane and beet molasses, in dried and liquid form, have high sugar contents; however, values are variable due to different processing methods and source of material (Hall, 2002). Cane molasses is readily available in tropic and sub-tropic areas and is a by-product derived from sugarcane (McDonald *et al.*, 2010). Molasses fermentation yields higher butyrate and lower propionate in the rumen (Hall, 2002; McDonald *et al.*, 2010).

2.4.1.5 Maize

Maize grain is an excellent source of digestible energy; however, it is relatively low in protein and NDF (McDonald *et al.*, 2010). The starch in maize is more slowly digested (4-6%/hour; Herrera-Saldana *et al.*, 1990), which is poorly matched to the more rapid degradation of the N in pasture (9-14%/hour; Van Vuuren *et al.*, 1991). The high starch content of maize could result in a lower ruminal pH due to the high production of VFA as well as lactate (Bach *et al.*, 1999).

Table 2-1 The chemical composition of some energy based feedstuffs (g/kg DM)

Parameter[2]	Dried citrus pulp[1]	Dried apple pomace[1]	Molasses (Sugar cane)[1]	Ground maize[1]
NFC	644	452	532	675-714
NSC	330	382	360	687-733
NDF	205-242	425-612	4	95-134
ADF	170-225	344-432	2	34
Lignin	9-21	150-178	0	9
Pectin	223	150-20	-	0
Starch	0-23	174	262	540-722
Sugar	125-402	208	98-540	20-40
ME (MJ/kg DM)	11.6-12.5	7.5-11.6	11.6	13.1
CP	41-94	56-80	58	94
EE	26-49	44-50	2	4
Ash	44-87	26	133	15

[1] Givens & Barber (1987); Edwards & Parker (1995); Knudsen (1997); Hall *et al.* (1998); Miller-Webster & Hoover (1998); NRC (2001); Hall (2002); Hindrichsen *et al.* (2004); Bampidis & Robinson (2006); Albuquerque *et al.* (2007); Abdollahzadeh *et al.* (2010); Mirzaei-Aghsaghali *et al.* (2011).
[2] NFC–Non-fibre carbohydrates; NDSF–Neutral detergent soluble fibre; NDF–Neutral detergent fibre; ADF–Acid detergent fibre; NSC–Non-structural carbohydrates; ME–Metabolisable energy; CP–Crude protein.

2.4.2 Specification for use in diets

The main source of NFC in the dairy industry is in the form of maize silage or maize grain, of which starch comprises 70-80% of the NFC fraction (NRC, 2001). Alternative energy sources include various by-products from the vegetable and fruit industry as well as from the animal feed industry itself. The use of these by-products, referred to as non-forage fibre source (NFFS), is impeded by the high fibre content and the simultaneously rapid passage through the rumen, much like with concentrate feeds (Bradford & Mullins, 2012). The use of NFFS products is further complicated by the variability of the product. Optimal inclusion levels of NSC and NFC are not well defined. It is recommended by the NRC (2001) that the NFC content of the ration for lactating Jersey cows should be 360-440 g/kg DM, with the NSC fraction included in that. In a study by Broderick *et al.* (2008) no adverse effects on rumen health and

milk production was recorded for diets containing 430 g/kg DM NFC and 310-330 g/kg DM NSC.

Pectins are fermented primarily to acetate and organic acids are not fermented in any measurable form. It is thought that NSC could provide a better estimation of carbohydrates fermented to propionate, carbohydrates contributing to microbial populations and the effect of carbohydrates on ruminal pH (Mertens, 1996). Changing the NFC content of the diet has been shown to affect rumen fermentation patterns, total tract digestion of fibre and milk fat content (Mertens, 1996; NRC, 2001; Bampidis & Robinson, 2006; Hindrichsen *et al.*, 2005).

In pasture-based systems forage NDF is essential for the stimulation of salivation and rumination, which helps maintain the pH of the rumen and prevent the onset of acidosis. High quality pastures are characterised as having 400-500 g/kg NDF and 180-250 g/kg CP, which indicates that they are more highly digestible and generally provide less peNDF (Bargo *et al.*, 2002; Bargo *et al.*, 2003; Plaizier *et al.*, 2009). High quality pastures combined with concentrate feeding do not provide adequate peNDF and as a result the pH of the rumen and the ratio of acetate to propionate decreases and the passage rate of feed increases (NRC, 2001; Bargo *et al.*, 2002). Pasture typically contains 50-300 g/kg DM NFC which is lower than the 350 g/kg DM recommended feeding level for lactating cows (Carruthers & Neil, 1997).

2.4.3 Production parameters

2.4.3.1 Milk yield

Milk yield depends primarily on the ME content provided. Secondly milk yield is dependent on microbial activity and the production of organic acids as end products of fermentation and degradation. The supplementation of a starch based concentrate provides high ME content and most often yields high milk production. Replacing starch with pectin and/or sugar in a concentrate feed influences milk production and milk composition in various ways. In a study by Broderick *et al.* (2008) starch was replaced incrementally with sucrose in a TMR ration, through the addition of molasses, without any detrimental effect on milk yield. Similarly, Leiva *et al.* (2000) also found no change in milk production for cows on a TMR system, where hominy chop was partially replaced with DCP. Cherney *et al.* (2003) added sucrose at 19 and 36 g/kg DM of the total diet without any increase in milk production. In a trial by Abdollahzadeh *et al.* (2010) apple pomace was ensiled with tomato pomace and included in a TMR, partially replacing lucerne hay and wheat bran. The tomato pomace was included due to its high CP content (217 g/kg DM) and its palatability compared to urea, thus making up for

the low CP content of apple pomace. It was found that at a 15% substitution of the apple pomace and tomato pomace mix, milk yield increased by 2 kg/d; however, there were no differences in 3.5% FCM yield.

The NFC source used in concentrate feeds affects milk production of cows consuming pasture by either causing a decrease in milk production or no change in milk production. Milk production has not been shown to increase in any study. The lower ME content of alternative sources in comparison to that of maize and the shift in VFA profile to more acetate and less propionate could be causative. Delahoy *et al.* (2003) found no change in milk production when ground maize was partially substituted with beet pulp in a concentrate fed to cows grazing medium quality pasture; different concentrates all had similar ME values. This was similar to results obtained by O'Mara *et al.* (1997), where the supplementation of beet pulp pellets to cows fed cut ryegrass did not have any effect on milk production. The addition of sucrose to the diet could lead to a decrease in milk production as was the case in a study by Higgs *et al.* (2013). Here molasses was fed in a liquid form, as a supplement to pasture. Milk yield was lowest for cows not receiving any grain based concentrate supplement; however, total ME intake was correspondingly lower for this group as well. When efficiency of production is considered it is possible that ME utilisation and efficiency was improved; however, it was not discussed.

2.4.3.2 Milk composition

Milk yield does not increase in response to higher sucrose inclusion in the diet (Oba, 2011); however, it has been shown, in several studies, that it has a positive effect on milk fat content (Broderick *et al.*, 2008; Nombekela & Murphy, 1995; Penner & Oba, 2009). Milk fat content is more sensitive to VFA production in the rumen and any digestive upsets can be identified rapidly. High levels of starch in the diet have a negative effect on ruminal pH and results in higher production of propionate, thus leading to lowered milk fat content, but higher milk yield. When starch is substituted with sucrose and/or pectin an increase in milk fat content can be expected, as was seen by Leiva *et al.* (2000) (TMR) and Higgs *et al.* (2013) (pasture) where milk fat content increased from 2.71 g/kg to 2.83 g/kg for Holsteins and from 3.88 g/kg to 4.57 g/kg for Friesian and Friesian x Jersey cows, respectively. Milk fat content increases in response to higher sucrose inclusion, due to the increased production of butyrate (Khalili & Huhtanen, 1991); however, the increase in milk fat content is not always accompanied by an increase of butyrate in the rumen (Broderick *et al.*, 2008). Acetate, along with butyrate, is an

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important precursor to de novo fatty acid synthesis for milk fat formation and should also be considered.

An increase in milk protein content is expected when starch is substituted by sucrose, due to the increase in microbial production (MP; Hall & Herejk, 2001). However, results have been variable, with no records of an increase in milk protein content. A decrease in milk protein content was recorded in a study where liquid molasses was used to substitute for grain based concentrate supplement for cows grazing ryegrass pasture (Higgs *et al.*, 2013). Similarly Delahoy *et al.* (2003) substituted ground maize with beet pulp (18 g/kg DM) in a concentrate fed to cows on pasture, which resulted in a decrease in milk protein content. Broderick *et al.* (2002) and Leiva *et al.* (2000) replaced cracked maize and hominy chop with dried citrus pulp at 190 g/kg DM and 210 g/kg DM of the total diet, respectively, resulting in a decrease in milk protein content. No change in milk protein was found by both McCormick *et al.* (2001) and Cherney *et al.* (2003), where sucrose was supplemented at 50 g/kg DM in the concentrate and at 19-36 g/kg DM in the total diet, respectively.

2.4.4 Rumen health and functionality

The fermentation of the various components of NFC differs from one another in digestion characteristics, specifically referring to the profile of organic acids produced (Strobel & Russell, 1986). Organic acid production depends on the amount of C, as a proportion of the molecular weight of monomers, which is available for use by rumen micro-organisms (Hall & Herejk, 2001). Therefore it is important to view rumen micro-organisms as separate entities when considering volatile fatty acids (VFA) production, fluctuations in the ruminal pH profile, influence on pasture DM and NDF degradability and the rumen microbial ecology.

2.4.4.1 VFA production

Total VFA. Various *in vitro* and *in vivo* studies have been done to investigate the effect of NFC source on VFA production, providing insight into microbial activity and efficiency of use. In general there is no response in total VFA production to different NFC sources provided *in vitro* (Ariza *et al.*, 2001; Mansfield *et al.*, 1994) and *in vivo* (Ben-Ghedalia *et al.*, 1989; Khalili & Huhtanen, 1991; Chamberlain *et al.*, 1993; Leiva *et al.*, 2000; Sannes *et al.*, 2002). However, an increase in total VFA production was reported by Bach *et al.* (1999) where cracked maize was substituted with beet pulp.

Acetate. Through *in vitro* studies it has been determined that the addition of pectin increases

the production of acetate expressed as a percentage of total VFA concentration; 84-95% compared to 56-71% when starch is fed (Ariza *et al.*, 2001; Hall, 2011). In an *in vitro* gas production study by Hatfield & Weimer (1995) the use of pectin from DCP yielded a higher acetate production, coupled with an overall increase in the acetate to propionate ratio, compared to other soluble substrates. In a continuous culture system the addition of sucrose to the diet increased acetate production (Ribeiro *et al.*, 2005). Similarly, the use of beet pulp over cracked maize in a pasture-based system increased the production of acetate (Bach *et al.*, 1999). Alternatively, McCormick *et al.* (2001) found no difference in acetate production when sucrose was supplemented *in vitro*. In an *in vivo* study by Ben-Ghedalia *et al.* (1989), an increase in acetate production, corresponding to an increase in the acetate to propionate ratio, was found in sheep fed a diet with 844 g/kg DM DCP. Poulsen *et al.* (2012) found an increase in total acetate production when pectin was used as an energy source over starch. In a study by Broderick *et al.* (2008) the addition of sucrose to a standard TMR resulted in a decreased acetate concentration and lower acetate to propionate ratio. In TMR feeding system, based predominantly on maize silage and lucerne hay, the addition of molasses to the diet did not influence acetate production or the acetate to propionate ratio (Oelker *et al.*, 2009).

Propionate. Higher acetate: propionate ratio of 4.1 was found when DCP was included in the ration compared to acetate: propionate ratio of 2.8 for hominy chop alone (Mertens, 1996; Ariza *et al.*, 2001). However, no change in propionate production was recorded when sucrose was supplemented *in vitro* (McCormick *et al.*, 2001). An *in vivo* study by Oelker *et al.* (2009) has shown that the addition of sucrose, through the means of molasses, did not affect propionate production.

Butyrate. Sugar has been shown to increase the production of butyrate in the rumen (Ribeiro *et al.*, 2005; Oba, 2011; Hall, 2011) and it is speculated that milk fat % increases as a result of this (Broderick *et al.*, 2008). Sucrose addition to the diet increased butyrate production in a continuous culture system (Ribeiro *et al.*, 2005); however, in a study by McCormick *et al.* (2001) no change in butyrate production was found when sucrose was supplemented *in vitro*. Butyrate production remained unchanged in an *in vivo* study where molasses was included to maize silage and lucerne based TMR feeds (Oelker *et al.*, 2009).

Branched chain VFA. Branched chain volatile fatty acids (BCVFA) are essential for the effective functioning of cellulolytic bacteria and the synthesis of microbial protein, specifically the amino acids Val, Ile, Leu and Pro (Cummins & Papas, 1985; Andries *et al.*, 1987). Isobutyrate and isovalerate production did not respond to the addition of molasses to maize

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based and lucerne based TMR feeds and no change was observed (Oelker *et al.*, 2009). No change in the BCVFA production was found when cracked maize was substituted with beet pulp, *in vitro* (Bach *et al.*, 1999). In a study by Broderick *et al.* (2008) an increase in sucrose content of the feed resulted in a decrease in isobutyrate and isovalerate concentration as well as in the total BCVFA concentration. Similar results were found by Ben-Ghedalia *et al.* (1989).

2.4.4.2 Ammonia nitrogen

Many rumen micro-organisms require NH_3-N for the synthesis of MP. The incorporation of NH_3-N into MP is an essential step in improving the N use efficiency of cows on pasture (Higgs *et al.*, 2013). As stated, the high soluble CP content of forages, coupled with lower than required WSC content could result in an excess production of NH_3-N, which cannot be incorporated into MP due to a lack of readily fermentable carbohydrates (Heldt *et al.*, 1999a; McCormick *et al.*, 2001). Rumen micro-organisms are tolerable to a wide range of NH_3-N levels, ranging from as low as 1-6 mg/dL (Hoover, 1986; Khalili & Sairanen, 2000) to as high as 80 mg/dL (Satter & Slyter, 1974). Rumen NH_3-N concentration normally ranges between 8.7-32.2 mg/dL, with a mean concentration of 18.3 mg/dL (Bargo *et al.*, 2003). A higher NH_3-N concentration in the rumen will allow for greater MP production, given that enough energy is readily available to rumen micro-organisms, as such it is found that increasing levels of NFC in the diet decreases the NH_3-N concentration due to improved N uptake by rumen micro-organisms (Bach *et al.*, 2005). If there is asynchrony of protein and energy supply to the rumen, excess NH_3-N, which cannot be incorporated in MP, will be taken up into the bloodstream and eventually converted to urea in the liver, an energy expensive process (Hristov & Broderick, 1996; McCormick *et al.*, 2001; Cajarville *et al.*, 2006). The MUN content in milk can be used as an indicator of excessive NH_3-N or poor incorporation of NH_3-N into MP (Jonker *et al.*, 1998). Cajarville *et al.* (2006) noted that the minimum NH_3-N concentration coincided with the maximum ruminal pH (r = -0.39, P <0.001). Cellulolytic bacteria derive N exclusively from NH_3-N (Russell *et al.*, 1992) and function most optimally when ruminal pH is above pH 6.0. Thus, higher ruminal pH, such as when more peNDF is available, could result in increased cellulolytic bacterial activity, leading to lower NH_3-N concentration. The production of NH_3-N in the rumen is influenced by the level of specific NFC included. The inclusion of molasses in the diet resulted in a quadratic increase in NH_3-N in the rumen of cows receiving a diet composed of predominantly lucerne- and maize- silage (Broderick & Radloff, 2004).

2.4.4.3   Ruminal pH

The biggest concern with supplemental feeding of cows on pasture is the effect of the concentrate on ruminal pH. Feeding starch generally leads to an increase in milk production; however, it leads to a decrease in ruminal pH, due to the production of lactate (Calsamiglia *et al.*, 2010; Poulsen *et al.*, 2012). The decreased ruminal pH results in lowered digestibility of pasture and poor N use efficiency (Calsamiglia *et al.*, 2010; Jacobs, 2014).

The rapid fermentation of sugar relative to other NFC sources would be expected to result in a lower ruminal pH (Oba, 2011); however, it has been shown in numerous *in vivo* and *in vitro* trials that this is not the case. Sugar provides less C for VFA production per unit of mass, compared to starch (Hall & Herejk, 2001) and increase the passage rate and production of MP (Sutoh *et al.*, 1996; Ribeiro *et al.*, 2005), essentially providing less OM for fermentation (Allen, 1997). Rumen micro-organisms are also able to convert sugar to glycogen for short term energy storage (Hall & Weimer, 2007), temporarily reducing VFA production in the rumen (Oba, 2011). Additionally, increased butyrate production, often also valerate production, from sugar fermentation decreases the production of protons per unit of ruminally degraded OM (Owens & Goetsch, 1988), thereby minimising the potential negative effect on ruminal pH. On TMR trials it has been found that the replacement of maize with DCP, yielding a diet with 430 g/kg DM NFC and 330 g/kg DM NSC, does not negatively affect ruminal pH (Broderick *et al.*, 2008). In a study by Broderick & Radloff (2004), where molasses was supplied in dry and liquid form to a predominantly lucerne hay and maize silage based diet, no negative effect on ruminal pH was experienced when sugar content was as high as 100 g/kg DM. Similarly, Leiva *et al.* (2000) and Oelker *et al.* (2009) found no change in ruminal pH when hominy chop was replaced with DCP in a TMR or when molasses was added to a maize silage- or lucerne hay-based TMR feed, respectively. The incremental substitution of maize with beet pulp (0-240 g/kg DM) in a TMR also did not cause a change in ruminal pH and time spent below pH 6.0 (Voelker & Allen, 2003).

Most of the studies investigating the use of pectin sources as an alternative to starch sources have been done in continuous culture systems where the pH of the culture has been managed to remain constant. In a study by Ariza *et al.* (2001) more HCl was needed to lower the pH of the culture fed DCP, indicating that a drop in pH might not be experienced. Bach *et al.* (1999) found no difference in pH when beet pulp and molasses was supplemented along with pasture compared to cracked maize in a continuous culture system. Pectin is degraded fairly rapidly in

the rumen, but it is different from starch in the sense that it is not fermented to lactate and in this manner it does not contribute much to the decline in ruminal pH (Strobel & Russell, 1986; Van Soest *et al.*, 1991; Hatfield & Weimer, 1995; Bampidis & Robinson, 2006). Also, pectin fermentation ceases under low ruminal pH, so unlike starches the fermentation of pectin does not result in a cumulative effect of lowered ruminal pH (Strobel & Russell, 1986; Allen, 2001).

2.4.4.4   Pasture DM and NDF degradability

Starch supplementation could lead to lowered ruminal pH, hindering NDF degradability of pasture, limiting pasture DM intake (DMI) and decreasing production from pasture (Jacobs, 2014). Penner & Oba (2009) found no effect on apparent total tract DM, OM, NDF or starch degradability when dry, cracked maize was replaced by sucrose in a TMR, yielding a diet with NFC content between 334–345 g/kg DM. However, a linear increase in DM, OM, NDF and ADF degradability was found when high moisture shelled maize was substituted with dried molasses (Broderick & Radloff, 2004). The total sugar content of the diet ranged from 24 g/kg DM (0 g/kg DM molasses) to 72 g/kg DM (120 g/kg DM dried molasses). A quadratic effect on NDF degradability was observed when starch was replaced at up to 75 g/kg DM with sucrose, with peak degradability reached at 50 g/kg DM sucrose and 25 g/kg DM starch in the diet (Broderick *et al.*, 2008), indicating an upper limit to which sucrose should replace starch with regards to roughage degradability. In an *in vitro* study by Malan (2009) it was found that the use of maize meal, DCP and molasses as a substrate increased the DM degradability of ryegrass pasture as well as kikuyu pasture, with molasses having the largest improvement on DM degradation. No effect on NDF degradability was seen when maize meal, DCP or molasses was used as a substrate and NDF degradability was not increased compared to the control where no substrate was added (Malan, 2009). This is in contrast to Holtshausen (2004) where increasing level of sucrose and pectin resulted in a linear and quadratic increase in NDF degradation at 24 hours of incubation, respectively. The protein source provided plays a pivotal role in determining NDF degradability when different NFC sources are fed. Heldt *et al.* (1999b) found that when RDP was sufficient sucrose supplementation actually increased OM and NDF degradability, compared to starch supplementation for steers grazing poor quality pasture.

2.4.4.5   Microbial community composition

The complex community of micro-organisms in the rumen are responsible for the breakdown of feed components, providing up to 70% of the ME required by the ruminant

animal as a by-product (Bergman, 1990; Weimer *et al.*, 1999). The composition and activity of rumen micro-organisms is significantly affected by modifications to the diet (Broadway *et al.*, 2012; Poulsen *et al.*, 2012; Hernandez-Sanabria *et al.*, 2012) and is influenced by the complexity of available substrates within different feeds (Welkie *et al.*, 2010; de Menezes *et al.*, 2011). Furthermore, ruminal bacterial community dynamics are influenced by diurnal variations in animal metabolism (Dehority & Orpin, 1997), cow breed (Guan *et al.*, 2008), individual cow variation (Weimer *et al.*, 1999; Welkie *et al.*, 2010) and stage of lactation and parity (Jewell *et al.*, 2015). Quantitative information regarding the effect of diet on the ruminal bacterial community is still lacking and has mostly been based on assumptions derived from culture dependent population estimates and correlation with fermentation end products and accumulation (Palmonari *et al.*, 2010; Broadway *et al.*, 2012). Rumen acidosis and methanogenesis are two examples that highlight the significant interaction between diet, rumen bacterial metabolism and ultimately, production output (de Menezes *et al.*, 2011). It has been shown on multiple occasions that high fibre diets increase methanogenesis in the rumen (Poulsen *et al.*, 2012), which is detrimental to the environment. However, the use of high fibre by-products, especially products previously labelled as 'waste' is increasing, potentially leading to an increase in methanogenesis. Through the use of technologies, such as Next-generation sequencing, it is possible to obtain much greater coverage of bacterial diversity and physiology (Hall, 2007; Buddle *et al.*, 2011), especially when in reference to two separate issues that seem to be working against each other. Alternatively, it has been shown that methane emissions decrease when cows are fed a pasture-based diet, compared to TMR systems (Buddle *et al.*, 2011). The cost of supplementation of high fibre by-products to cows on pasture on methane emissions will depend on specific bacterial communities and their prevalence under these different feeding strategies, finding a compromise between the two issues.

Ruminal bacterial diversity is most commonly presented at a genus level, as genera shifts are more representative of changes in the bacterial community dynamics in response to diet changes (Broadway *et al.*, 2012). The ruminal bacterial community is extremely diverse, but only a few genera dominate the population, most notable *Prevotella* and *Eubacterium*. *Prevotella* are able to utilise a variety of nutrients to survive and sustain growth and have been shown to be the predominant bacteria in the rumen (Tajima *et al.*, 2001; Stevenson & Weimer, 2007). *Eubacterium* are the second most dominant bacteria in the rumen, fermenting pyruvate and amino acids it is most important for cows consuming a high protein diet (Broadway *et al.*, 2012). The remaining portion of the bacterial community does respond to changes in the diet

by shifting in population density (Broadway *et al.*, 2012); however, they make up such a small part of the total bacterial community, the significance of their population shifts is not fully understood (Palmonari *et al.*, 2010). Furthermore, a loss of minority community members is often seen instead of a dramatic shift in the composition of the microbiome (Broadway *et al.*, 2012). Ruminal bacterial diversity is also often viewed on a phylum level, with the major part of the population being made up of *Firmicutes* and *Bacteroidetes* (Stewart *et al.*, 1997; Jewell *et al.*, 2015). There are two distinct 'areas' of bacterial inhabitation in the rumen, namely the liquid and solid phases of rumen digesta (Weimer *et al.*, 1999; Pitta *et al.*, 2010). In a study by de Menezes *et al.* (2011) the difference in these two populations on both TMR and pasture base diets was shown, with distinct community composition differences between the liquid and solid phase digesta and between the TMR and pasture-based diets. This was similar to observations of Welkie *et al.* (2010), where the microbial population differed between the solid and liquid phases of rumen digesta.

Observed ruminal bacterial community dynamics changes between various cows or treatments (such as diet type) are not always accompanied by different metabolic outputs. This suggests that there is considerable niche overlap and even capacity for niche replacement within the ruminal microbial community (Fernando *et al.*, 2010; Palmonari *et al.*, 2010; Welkie *et al.*, 2010). It was posited by Jewell *et al.* (2015) that functional role, rather than taxonomic identity, drives the composition of the ruminal bacterial community, especially during lactation. Generally there has been a lack of research investigating the correlation between the cows' biology (phenotype) and the rumen bacteria (Guan *et al.*, 2008; Callaway *et al.*, 2010). Therefore, it cannot be assumed that the ruminal bacterial community dynamics were not affected by experimental procedure, solely due to the fact that there was no change in the ruminal diurnal pH fluctuations and VFA production.

Dehority (1969) identified the ability of several strains of rumen associated micro-organisms to utilise galacturonic acid as an energy source, suggesting that species differences are present in the enzymatic degradation of pectin. Bacterial community dynamics was investigated by Poulsen *et al.* (2012) with the use of terminal-restriction fragment length polymorphism analysis (T-RFLP). It was found that the bacterial community responsible for the fermentation of pectin did not overlap with the bacterial community responsible for the fermentation of wheat and maize starch. This is indicative of a selective promotion of groups of rumen associated bacteria utilising pectin as a substrate for growth (Poulsen *et al.*, 2012). Bacteria that are able to utilise pectin include *Fibrobacter succinogenes*, *Prevotella ruminicola*,

Butyrivibrio fibrisolvens, *Streptococcus bovis*, *Bacteroides ruminicola*, *B. succinogenes*, *Succinivibrio dextrinosolvens* and *Lachnospira multiparus* (Czerkawski and Breckenridge, 1969; Dehority, 1969; Gradel and Dehority, 1972; Baldwin and Alisson, 1983). There are several amylolytic rumen bacteria involved in the fermentation of starch, including *Ruminobacter amylophilus*, *P. ruminicola*, *P. bryantii*, *Succinimonas amylolytica*, *S. bovis*, *Selenomonas ruminantium*, *B. fibrisolvens*, *Eubacterium ruminantium* and *Clostridium spp*. (Cotta, 1988; Tajima *et al.*, 2001). The primary rumen bacteria responsible for the fermentation of sugar are *S. bovis*, *L. multiparus*, *Lactobacillus ruminis*, *Lactobacillus vitulinis*, *Clostridium longisporum*, *Eubacterium cellulosolvens* and some strains of *E. ruminantium*, *B. fibrisolvens*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Megaspaera elsdenii*, *Prevotella spp.*, *Selenomonas ruminantium*, *S. dextrinosolvens* and *Pseudobutyrivibrio ruminis* strain A (Stewart *et al.*, 1997; Stan-Glasek *et al.*, 2010).

2.4.4.6 Microbial protein

The activity of micro-organisms is essential for the production of MP, which is an invaluable source of amino acids (Hall & Herejk, 2001). The rate of substrate fermentation is approximately proportional to the rate of microbial growth (Nocek and Russell, 1988); however, substrate disappearance or digestion does not necessarily mean that MP is being synthesised (Russell, 1998). In these terms sucrose is expected to yield the highest MP due to the rapid rate of fermentation and pectin and starch would yield lower levels, similar to each other (Russell *et al.*, 1992). However, this is not always the case. In a study by Hall & Herejk (2001) starch fermentation yielded more MP than sucrose and pectin. The lower MP production from sucrose could be as a result of less C that is available for MP synthesis (Hall & Herejk, 2001). It is also possible that the higher rate of fermentation of sucrose in comparison to starch, could lead to energy spilling, decreasing the energy available for MP synthesis (Strobel & Russell, 1986). This could provide an explanation for lowered milk protein content found in various *in vivo* trials where starch was substituted with pectin and sucrose, highlighting the fact that NFC type can alter MP yield and cow performance (Hall & Herejk, 2001). Microbial protein yield does not only depend on NFC source, but also on the presence of RDP and RUP (Mertens *et al.*, 1994).

2.4.5 Pasture DM intake

The feeding of a concentrate supplement to cows on pasture results in lowered DMI of

pasture and is referred to as substitution rate (Stockdale, 2000; McEvoy *et al.*, 2008). On a pasture-based system maximum pasture DMI is essential and substitution rate should be considered when deciding on a concentrate supplement to be fed. In a study by Delahoy *et al.* (2003) the partial substitution of maize with beet pulp did not have any effect on pasture DMI and did not result in an increase in substitution rate. Higgs *et al.* (2013) estimated pasture DMI of cows receiving diets with a starch and sucrose content of 3 g/kg DM and 218 g/kg DM or 165 g/kg DM and 102 g/kg DM, respectively. Pasture DMI was highest for cows receiving only molasses, with no additional concentrate supplement and pasture also made up 87% of the diet compared to a high starch diet where pasture only made up 72% of the total daily DMI. When pasture DMI of cows on different treatments was compared to pasture DMI of the control group it is seen that both the feeding of molasses and the feeding of a high fibre concentrate promoted pasture DMI and did not result in any pasture substitution. The high starch diet resulted in substitution rate of 0.3-0.45 kg for every 1 kg concentrate fed. Replacing 60% of the maize in a concentrate with DCP and molasses had no effect on pasture DMI, as estimated with Cr_2O_3 (Gehman *et al.*, 2006). However, the Cr_2O_3 method for determining pasture DMI has been shown to be inaccurate due to partial faecal recovery of Cr_2O_3 and variation between individual cows (Titgemeyer *et al.*, 2001), warranting the application of more modern techniques.

In a TMR system DMI increases linearly in response to higher sucrose inclusion in the diet at the expense of starch, with the increased consumption attributed to the improved palatability of the diet (Bampidis & Robinson, 2006; Broderick *et al.*, 2008; Penner & Oba, 2009). Total DMI does not always respond to supplementation with sugar, with no change in DMI found by McCormick *et al.* (2001) and Penner *et al.* (2009).

2.4.6 Nitrogen utilisation

Supplementing starch with sucrose could benefit N metabolism and utilisation; however, there are many confounding findings. Various studies have improved N use efficiency; there was a decrease in urinary N excretion (Sutoh *et al.*, 1996; Sannes *et al.*, 2002; Broderick & Radloff, 2004; Broderick et al, 2008), an increase in N retention (Sutoh *et al.*, 1996) and improved microbial protein synthesis (Ribeiro *et al.*, 2005). Other studies have shown a decrease in N utilisation efficiency; increase in plasma urea N (McCormick et al, 2001; Penner & Oba, 2009) and a decrease in milk protein as level of starch supplemented with sucrose increased (Broderick *et al.*, 2008). The rate of ryegrass N degradation in the rumen is estimated

at 9-14%/h (Beever *et al.*, 1986), whereas the rate of degradation of the carbohydrates (mostly structural) is slower at only 7%/h (Van Vuuren *et al.*, 1991). Starch in maize degrades at a rate of 4-6.4%/h (depending on grain processing) (Herrera-Saldana *et al.*, 1990), whereas pectin in DCP degrades at a rate of 13%/h (Hall *et al.*, 1998), thus providing better synchrony of N and energy metabolism (Gehman *et al.*, 2006; Higgs *et al.*, 2013). The better synchrony of N and energy metabolism could result in improved N use efficiency and improved N utilisation. Improved microbial protein synthesis has been observed when feeding DCP (Ariza *et al.*, 2001; Bampidis & Robinson, 2006) and when supplementing sucrose (Ribeiro *et al.*, 2005), once again indicating improved microbial activity and improved degradation of pasture. Blood urea N and MUN values are positively correlated with rumen NH_3 content (DePeters and Furgeson, 1992). Therefore, blood urea nitrogen and MUN can be effectively used as indicators of N use efficiency.

In a study by Higgs *et al.*, (2013) the effect of various energy based supplements on N use efficiency was tested. Lower urinary N was recorded for cows receiving only molasses as a supplement to pasture, compared to cows receiving only pasture; however, the total CP intake was lower for cows receiving molasses, as such there was no improvement in N use efficiency, rather only an N dilution effect. The substitution of starch with sucrose by Broderick *et al.* (2008) resulted in an improvement in the markers for N use efficiency (urinary urea-N, total N and urea N); however, there was no increase in milk protein secretion. Milk urea N values are highly variable depending on stage of lactation, milk yield and change in body weight (Kohn, 2007). The recommended range for MUN concentration is 8-12 mg/dL for samples collected from a bulk tank and 8-25 mg/dL for individual cows (De Villiers *et al.*, 2000; Kohn, 2007).

2.4.7 Methane gas production

Feeding starch as the main energy component in the diet could decrease methane gas emissions, but at the expense of rumen health and N use efficiency. The fermentation of sucrose and pectin habitually increases the production of hydrogen and carbon dioxide, substrates for methanogens (Van Kessel and Russell, 1996). This could possibly lead to an increase in methanogenesis, compared to starch (Van Kessel and Russell, 1996). In an *in vitro* study by Poulsen *et al.* (2012) it was found that pectin as a substrate had lowered methanogenesis than maize and wheat, opposite to what was expected. The *in vitro* system used in the study was regulated to maintain a constant pH, thus avoiding the drop in pH usually seen when feeding starch (maize and wheat), which leads to lowered methanogenesis. When the drop in pH is not

taken into consideration pectin is not expected to increase the level of methane gas production above the level of methane gas produced from starch. Alternatively, Hindrichsen *et al.* (2004) found that sugar could possibly increase methane gas production (*in vitro*), compared to starch, under conditions of high ruminal pH. In both instances, pH plays a more pivotal role in methanogenesis than the energy source. However, detailed carbohydrate analysis of feed is required to predict methane gas emissions from digestion (Hindrichsen *et al.*, 2005).

2.5 Conclusion

This literature review has confirmed that although extensive research pertaining to the use of alternative carbohydrate sources has been carried out over the last century, the use of alternative carbohydrate sources, specifically those derived from fruit and vegetable wastes, have not received as much attention. Furthermore, the application of alternative carbohydrates sources for cows in a grazing system have not been investigated intensively. Pasture-based dairy systems are the most popular form of dairy farming in South Africa and it is clear that more information is needed regarding this matter.

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Chapter 3: Replacing maize grain with dried citrus pulp in a concentrate feed for Jersey cows grazing ryegrass pasture

Abstract

Dried citrus pulp (DCP) is a high fibre by-product of the citrus industry. In total mixed ration systems it has been shown to maintain a more stable ruminal environment, improving overall production compared to maize. The aim of the study was to determine the effect of stepwise substitution of ground maize for DCP in a concentrate supplement on milk yield, milk composition and rumen health of Jersey cows grazing ryegrass pasture. Sixty-eight lactating Jersey cows ($\mu \pm SD$; 84.5 ± 43.8 days in milk, 20.4 ± 3.09 kg/day) were used in the trial. Cows were allocated to one of four treatments, with 17 cows per treatment. Treatments were: No DCP (NDCP)-0% replacement, Low DCP (LDCP)-33% replacement, Medium DCP (MDCP)-66% replacement and High DCP (HDCP)-100% replacement. An additional six ruminally cannulated Jersey cows were randomly allocated to the NDCP and HDCP treatments in a two period cross-over design. Milk yield decreased between 2.1 and 3.2 kg/day when ground maize was substituted by DCP. Milk fat content did not differ between treatments; however, treatment had a quadratic effect on milk protein and lactose content, with the LDCP and MDCP treatments having the highest values. No change in the diurnal ruminal pH curve and no differences in the rate and extent of pasture dry matter and neutral detergent fibre degradability between treatments were observed. In conclusion, replacing ground maize with DCP in a conventional concentrate diet led to a decrease in milk yield, while rumen health was maintained.

3.1 Introduction

In pasture-based dairy systems the use of supplemental concentrate feeding is essential to maximise milk production from pasture. Cereal grains form the largest part of the concentrate supplement and play an important role in determining the profitability of a dairy farm (Allen & Knowlton, 1995; NRC, 2001). The high starch content of cereal grains has a limiting effect on microbial activity in the rumen due to lactic acid production, resulting in low ruminal pH (Calsamiglia *et al.*, 2010; Poulsen *et al.*, 2012; Jacobs, 2014). A low ruminal pH impacts fibre degradation and has various negative production implications. Despite the problems associated with feeding starches it is still practised widely due to the high energy content, which promotes milk production. Other non-fibre carbohydrates (NFC), such as sugar and pectin (prevalent in various fruit wastes), have a more positive effect on the rumen environment (Hall & Herejk, 2001; Ribeiro *et al.*, 2005) and are able to maintain production when substituted in total mixed ration (TMR) systems (Leiva *et al.*, 2000; Broderick *et al.*, 2008). Dried citrus pulp (DCP) is a by-product of the juicing industry (Bampidis & Robinson, 2006) and it is high in sugar (208 g/kg dry matter; DM) and pectin (150-200 g/kg DM) and low in starch (174 g/kg DM) (Miller-Webster & Hoover, 1998; Hindrichsen *et al.*, 2004). The inclusion of DCP in dairy rations is common practise for TMR systems, where it acts as a flavour enhancer due to the high sugar content, promoting feed intake (Bampidis & Robinson, 2006; Penner & Oba, 2009, Oba, 2011). No previous work has been documented on the use of DCP as a feed source for cows grazing pasture. Citrus pulp is a seasonal feed source; however, there is merit in investigating the use of this feed source in South Africa as it could offer a possible alternative to maize at times when the maize cost is high. It is often used as is, but in a dried form it can be stored long term, forming an integral part of the annual feeding plan of a farm. The aim of the study was to determine how effectively ground maize could be replaced with DCP in a concentrate supplement fed to Jersey cows grazing annual ryegrass pasture and what the possible rumen health implications would be.

3.2 Materials and methods

The trial was carried out on the Outeniqua Research Farm, George, South Africa (22º 25' 16" E and 33º 58' 38" S) during early spring, September to November, of 2013. The mean minimum and maximum temperatures were 11.7 °C and 21.5 °C, respectively, and the total rainfall during the trial period was 295 mm (Agricultural Research Council, 2013). Kikuyu

pasture (*Pennisetum clandestinum*) had been over-sown with annual Italian ryegrass (*Lolium multiforum* var. Italicum, cv. Jeanne) on an 8.6 ha area under permanent irrigation during the previous autumn. An Aitcheson seeder was used for seeding at a rate of 18 kg/ha. The area used was characterised by a Witfontein soil form (Swanepoel *et al.* 2013) and paddocks were fertilised with 42 kg N/ha using limestone ammonium nitrate (280 g N/kg) after each grazing. Ethical clearance was obtained through the Western Cape Department of Agriculture, South Africa, clearance number: R13/83. Experimental treatments were defined according to the level of ground maize in the concentrate supplement that was replaced by DCP. Treatments were: No DCP (NDCP) denotes 0% replacement, Low DCP (LDCP) denotes 33% replacement, Medium DCP (MDCP) denotes 66% replacement and High DCP (HDCP) denotes 100% replacement. The ingredient composition of the four concentrate supplements is shown in Table 3-1.

Table 3-1 Ingredient composition (g/kg) of the four concentrate supplements fed to sixty-eight Jersey cows grazing ryegrass pasture.

Parameter	Treatment[*]			
	NDCP	LDCP	MDCP	HDCP
Ground maize	750	500	250	0
Dried citrus pulp	0	250	500	750
Soybean oilcake	76	98	121	149
Wheat bran	94	81	67	38
Molasses (liquid)	40	40	40	40
Feed lime	20	10	0	0
Salt	10	10	10	10
Premix[**]	5	5	5	5
MgO	3	3	3	3
Mono-CaP	2	3	4	5

[*] NDCP-No dried citrus pulp, 0 % replacement; LDCP-Low dried citrus pulp, 33% replacement; MDCP-Medium dried citrus pulp, 66% replacement; HDCP-High dried citrus pulp, 100% replacement.
[**] Premix-4 mg/kg Cu; 10 mg/kg Mn; 20 mg/kg Zn; 0.34 mg/kg I; 0.2 mg/kg Co; 0.06 mg/kg Se; 6 x 10^6 IU vitamin A; 1 x 10^6 IU vitamin D$_3$; 8 x 10^3 IU vitamin E.

The trial consisted out of two components, namely the production study and the rumen metabolism study, which ran congruently with each other. The production study consisted of sixty-eight lactating Jersey cows that were blocked according to the average milk yield of the three weeks preceding the trial ($\mu \pm SD$; 20.4 ± 3.1 kg/day), days in milk (84.5 ± 43.8 days) and lactation number (4.5 ± 2.4). Cows within blocks were then assigned to treatments according to a complete randomised block design for a continuous lactation trial over a period of 51 days. The rumen metabolism study consisted out of six ruminally cannulated cows that were randomly allocated between the NDCP and HDCP treatments and subjected to a two-period cross-over design. A 14 day adaptation period was implemented after each cross-over,

before rumen data collection commenced. Cows in both the production study and the rumen metabolism study received 6 kg DM/day of the respective concentrate supplement, which was fed over two sessions, during the morning and afternoon milking. The area used for grazing was divided into 35, 0.25 ha paddocks, with an average grazing interval of 24 days. A rising plate meter (RPM; Jenquip, Reid Line East, RD 5 Feilding, New Zealand, 4775) was used to determine pasture height per paddock one day prior to grazing (Table 3-2). Pasture yield could then be estimated and allocated to cows by means of strip grazing. All cows grazed together and water was available *ad libitum* in the pasture camps.

Table 3-2 Mean (± SD) of the pre- and post-grazing RPM height, pasture yield, pasture allowance and pasture DM intake determined using the seasonal linear regression.

Parameter[*]	Pasture values
Pre-grazing	
RPM height	42.2 ± 8.93
Pasture yield (kg DM/ha)[**]	3640 ± 814
Pasture allowance (kg DM/cow/day)	11.2 ± 2.00
Post-grazing	
RPM height	13.0 ± 2.35
Pasture yield (kg DM/ha)	987 ± 214
Estimated pasture DM intake (kg/cow/day)	10.6 ± 1.85

[*] RPM-Rising plate meter; DM-Dry matter.
[**] $Y = 91.06*H\text{-}200.59$, where Y = DM yield and H = RPM reading (Steyn, 2012).

Cows were milked twice daily at 05:30 and 13:30, and milk yield was automatically recorded at each milking (Dairy Master). Composite milk samples, of the morning and afternoon milking sessions, were collected once every second week, preserved with Bronopol and analysed for fat, protein, lactose, milk urea nitrogen (MUN), somatic cell count (SCC) and pH using the FOSS CombiFoss[TM] FT+ (FOSS, Foss Allè 1, DK-3400 Hillerød, Denmark). The 4% fat corrected milk (FCM) yield was determined using the Gaines formula (Gaines, 1928), where 4% FCM = (0.4*kg milk) + (15*kg fat) to correct milk yield to a constant energy basis. Cows were weighed and scored at the commencement and completion of the trial. Body weight (BW) was recorded over two days and the average used to compensate for possible differences due to defaecation, urination and water intake. Body condition scoring (BCS) was performed using the five point scale described by Wildman *et al.* (1982) and Edmonson *et al.* (1989), where a score of one indicates an extremely thin cow and a score of five indicates an extremely fat cow. Average daily gain (ADG) was calculated as the weight difference obtained over a period of 51 days.

TruTrack pH Data Loggers (Model pH-HR mark 4, Intech Instruments LTD, New Zealand, www.intech.co.nz) were used to record the diurnal fluctuations of ruminal pH every 10

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minutes, over a 72 hour period. Loggers were carefully calibrated with two buffer solutions (pH 4 and 9) before insertion into the rumen. Rumen fluid samples were collected from ruminally cannulated cows at six-hour intervals (08:00, 14:00, 20:00 and 02:00) for analysis of ammonia nitrogen (NH$_3$-N; Broderick & Kang, 1980) and volatile fatty acids (VFA; Siegfried *et al.*, 1984). Samples were collected using a hand held suction pump. After collection samples were filtered through 4 layers of cheese cloth. Aliquots were then frozen at -20˚C in small air tight containers, until further analysis. Pasture DM and neutral detergent fibre (NDF) degradability was determined through the use of *in sacco* Ankom Dacron bags (10x20 cm), with a nominal average pore size of 53 µm, containing dried and cut (5 mm) pasture samples. Seventeen bags were prepared for each cow and bags were incubated for 2, 4, 8, 16, 30, 72 and 96 hours. Pasture residue in bags was analysed for DM (AOAC, 2002; method 934.01) and NDF (Robertson & van Soest, 1981; using the Ankom fibre analysis system; 71 Ramachandra Agrahara, Azad Nagar, Chamrajpet, Bangalore, 560 018). The pasture DM and NDF degradability was determined with the equation: $p = a + b\left(1 - e^{-ct}\right)$, where p = actual degradation at time t, a = intercept of degradation curve at $t = 0$, b = potential degradability of component, e = the base of natural logarithms and c = rate constant for degradation of coefficient b (Ørskov & McDonald, 1979).

Pasture and feed samples were collected once every second week, dried, and analysed for DM, organic matter (OM; AOAC, 2002; method 942.05), crude protein (CP; AOAC, 2002; method 990.03; using the Leco N analyser, model FP 528), NDF (Robertson & Van Soest, 1981; using the Ankom fibre analysis system with heat stable α-amylase, followed by incineration of the residue), acid detergent fibre (ADF; Robertson & Van Soest, 1981; using the Ankom fibre analysis system followed by incineration of the residue), neutral detergent insoluble nitrogen (NDIN; NDF procedure, residue analysed for CP), acid detergent insoluble fibre (ADIN; ADF procedure, residue analysed for CP), ether extract (EE; AOAC, 2002; method 920.39), gross energy (GE; MC 1000 Modular Calorimeter, Energy Instrumentation, Sandton, South Africa, 2146), *in vitro* organic matter degradability (IVOMD; Buys *et al.*, 1996), Ca and P. Metabolisable energy (ME) was calculated with the equation of Robinson *et al.* (2004): $ME\,(MJ/kg\,DM) = GE \times IVOMD \times 0.82$ (Robinson *et al.*, 2004). The NFC content was calculated as follows: $NFC\,(g/kg\,DM) = 100 - (NDF + CP + EE + Ash)$ NFC (g/kg DM) (NRC, 2001).

Milk yield, milk composition, BW and BCS data were subjected to a mixed model

procedure, using SAS version 9.2 (SAS, 2008). Polynomial contrasts were used to test for linear, quadratic and cubic effects of replacing ground maize with DCP at increasing levels. Covariance was not included due to the blocking of cows, which was expected to minimise variation based on cow factors, between treatments. Volatile fatty acid and NH_3-N data were analysed using a mixed model procedure over time. The *in sacco* Dacron bag study was subjected to a main effects ANOVA. The data were fitted to the non-linear model, $p = a + b(1 - e^{-ct})$, using an iterative regression analysis to determine the constants *a*, *b* and *c* (Ørskov & McDonald, 1979). The ruminal pH data were subjected to a repeated measures ANOVA. Tukey's test was used to compare the treatment means at a 5% significance level. The null hypothesis was: $H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_a$. The null hypothesis was rejected where $P < 0.05$ and a trend identified where $0.05 < P < 0.10$. Least squares means were used to calculate a pooled standard error of treatment means. Shapiro-Wilk tests were used to test for normality (Shapiro & Wilk, 1965).

3.3 Results

The HDCP concentrate supplement had a higher inclusion of soybean oilcake to compensate for the low CP content of the DCP. All four concentrate supplements were formulated to be iso-nitrogenous, although the final products differed slightly (Table 3-3). The level of mono-CaP had to be increased as the level of DCP increased to counteract the high Ca content of DCP, thus maintaining the Ca:P ratio. The same applies for the feed lime, which was highest in the NDCP concentrate supplement. The NDCP concentrate supplement had the highest NFC content and consequently the lowest NDF content. The ME content was similar in all concentrate supplements. Daily pasture DMI was estimated at 10.6 kg DM/cow, calculated as the difference in pasture yield before and after grazing, 3640 kg DM/ha and 987 kg DM/ha, respectively (Table 3-2). Pasture was grazed to an average of 13 on the RPM, which is slightly under-grazed. A reading of 10-12 on the RPM is indicative of a well grazed pasture (Irvine *et al.*, 2010); however, 13 on the RPM is not extreme and would imply that cows received enough pasture.

The inclusion of DCP in the ration, at the expense of ground maize, resulted in a linear decrease in milk yield and 4% FCM yield (Table 3-4). The respective decreases in milk yield for a 33, 66 and 100% replacement of ground maize with DCP compared to 0% replacement were 2.13, 2.27 and 3.23 kg/day. Differences in milk yield between the LDCP, MDCP and

HDCP treatments were not significant. Treatment also did not have an effect on milk fat content or SCC. The replacement of ground maize had a quadratic effect on protein and lactose content (P = 0.011 and 0.035, respectively). The initial replacement of ground maize with DCP, namely 33 and 66%, resulted in an increase in protein and lactose content, but thereafter, these two parameters decreased again. The MUN content of milk increased linearly with an increase in DCP inclusion level (P = 0.023). Treatment also had a linear effect on fat and protein yield (P <0.001), with fat and protein yield decreasing as the level of ground maize replaced with DCP increased. No linear, quadratic or cubic effects were observed for BW, ADG and BCS change.

At 14:30 the ruminal pH of cows in the HDCP treatment reached a lower level (P = 0.04) than that of cows in the NDCP treatment, pH 5.95 and pH 6.20, respectively (Figure 3-1). No other differences in pH were observed over the 24 hour period. A sudden and sharp decrease in ruminal pH was observed between 05:30-06:30 and between 13:30-14:30, which corresponded with consumption of the concentrate supplement in the milking parlour. Treatment did not have an effect on the daily mean ruminal pH (Table 3-5). There were also no differences in the duration of the ruminal pH below pH 6.2, pH 6.0 and pH 5.8. No difference in the total VFA concentration between cows in the NDCP and HDCP treatments was found (Table 3-5). The molar proportion of acetate, propionate, butyrate and valerate and the acetate to propionate ratio also remained unchanged. Isobutyrate (P = 0.04) and isovalerate (P = 0.02) concentrations were higher for cows in the NDCP treatment. The ruminal NH_3-N concentration was higher (P <0.01) for cows fed the HDCP concentrate supplement than cows fed the NDCP concentrate supplement at 02:00, 14:00 and 20:00 (Figure 3-2). No difference in ruminal NH_3-N concentration was observed at 08:00. There were no differences in the degradability of pasture DM and NDF at any of the incubation times (Figure 3-3 and Figure 3-4). All degradability parameters were the same for cows in the NDCP treatment and cows in the HDCP treatment (Table 3-5).

Table 3-3 Chemical composition (g/kg DM; mean ± SD) of the four concentrate supplements, the dried citrus pulp (DCP) used in the concentrate supplements and the ryegrass pasture (n = 5).

Parameter[*]	Treatment[**]				DCP	Pasture[***]
	NDCP	LDCP	MDCP	HDCP		
DM	892 ± 6.42	889 ± 8.12	894 ± 5.70	902 ± 8.72	870 ± 33.2	158 ± 21.0
OM	935 ± 1.47	930 ± 1.44	912 ± 3.46	894 ± 1.21	919 ± 3.58	884 ± 13.8
CP	117 ± 1.30	120 ± 2.91	117 ± 2.40	121 ± 2.42	48.1 ± 1.67	174 ± 26.2
EE	27.9 ± 6.14	26.8 ± 3.45	23.5 ± 2.98	18.1 ± 2.48	16.9 ± 2.04	41.5 ± 1.96
NFC	671 ± 15.6	607 ± 24.9	609 ± 14.5	581 ± 6.41	653 ± 3.08	231 ± 54.1
NDF	119 ± 20.0	168 ± 24.8	162 ± 12.2	174 ± 3.04	201 ± 7.02	438 ± 18.6
ADF	39.7 ± 3.34	96.5 ± 7.99	147 ± 4.48	198 ± 9.45	257 ± 14.2	298 ± 10.0
NDIN	5.86 ± 0.94	6.81 ± 1.30	8.01 ± 0.50	9.09 ± 0.20	7.45 ± 0.65	10.6 ± 2.23
ADIN	16.2 ± 4.72	8.02 ± 1.53	6.27 ± 0.66	5.21 ± 0.53	3.46 ± 0.32	5.15 ± 2.08
IVOMD	976 ± 29.7	980 ± 41.6	994 ± 30.7	993 ± 30.8	987 ± 5.37	872 ± 11.8
GE (MJ/kg DM)	17.1 ± 0.86	17.1 ± 1.01	16.6 ± 0.67	16.2 ± 1.15	16.0 ± 0.11	17.3 ± 0.88
ME (MJ/kg DM)	14.0 ± 3.61	14.0 ± 5.20	13.9 ± 3.74	13.5 ± 3.29	13.2 ± 0.02	12.7 ± 1.38
Ca	11.3 ± 0.17	13.1 ± 0.49	19.4 ± 6.22	25.2 ± 8.90	15.3 ± 1.53	3.91 ± 0.47
P	5.01 ± 0.08	4.72 ± 0.15	4.37 ± 0.11	4.31 ± 0.20	1.27 ± 0.21	5.61 ± 0.60
Mg	3.62 ± 0.16	3.39 ± 0.20	3.32 ± 0.05	3.28 ± 0.17	1.21 ± 0.04	3.31 ± 0.21
K	9.4 ± 0.17	10.9 ± 0.32	12.6 ± 0.17	14.5 ± 0.22	9.59 ± 0.37	36.0 ± 12.5

[*] DM-Dry matter; OM-Organic matter; CP-Crude protein; EE-Ether extract; NFC-Non-fibrous carbohydrates; NDF-Neutral detergent fibre; ADF-Acid detergent fibre; NDIN-Neutral detergent insoluble nitrogen; ADIN-Acid detergent insoluble nitrogen; IVOMD-In vitro organic matter degradability; GE-Gross energy; ME-Metabolisable energy.
[**] NDCP-No dried citrus pulp, 0 % replacement; LDCP-Low dried citrus pulp, 33% replacement; MDCP-Medium dried citrus pulp, 66% replacement; HDCP-High dried citrus pulp, 100% replacement.
[***] Pasture-Annual Italian ryegrass (*Lolium multiforum*, variety *Italicum*, cultivar Jeanne).

Table 3-4 Mean milk yield, milk composition and body weight and body condition score change of cows receiving one of four concentrate supplements (n = 17).

Parameter[*]	Treatment[**]				SEM	Linear	Quadratic	Cubic
	NDCP	LDCP	MDCP	HDCP				
Milk yield (kg/cow)	21.1[a]	19.0[b]	18.9[b]	17.9[b]	0.57	<0.001	0.140	0.110
4% FCM yield (kg/cow)	22.5[a]	20.3[b]	20.0[b]	19.4[b]	0.55	<0.001	0.103	0.272
Fat (g/kg)	44.8	44.9	44.5	45.6	1.13	0.685	0.616	0.716
Protein (g/kg)	34.9[ab]	35.8[a]	35.6[a]	34.4[b]	0.56	0.352	0.011	0.956
Lactose (g/kg)	46.5[a]	47.0[a]	47.0[ab]	45.6[b]	0.30	0.638	0.035	0.864
SCC (x 10³ cells/mL)	93.8	200	208	173	40.2	0.146	0.061	0.745
MUN (mg/dL)	9.32[a]	9.38[a]	10.3[bc]	10.1[ac]	0.35	0.023	0.647	0.146
Fat yield (kg/cow)	0.94[a]	0.85[b]	0.83[b]	0.81[b]	0.02	<0.001	0.149	0.480
Protein yield (kg/cow)	0.73[a]	0.68[b]	0.67[b]	0.61[c]	0.02	<0.001	0.909	0.204
BW before (kg)	398	386	404	396	9.63	0.740	0.817	0.198
BW change (kg)	+12.3	+8.62	+8.50	+7.38	3.43	0.293	0.684	0.746
ADG (kg/d)	0.25	0.17	0.17	0.15	0.07	0.293	0.684	0.746
BCS before (scale 1–5)	2.15	2.15	2.13	2.16	0.05	0.888	0.754	0.779
BCS change (scale 1–5)	+0.22	+0.18	+0.24	+0.19	0.03	0.844	1.000	0.173

[a,b] Row means with different superscripts differ significantly at P <0.05.
[*] FCM-Fat corrected milk; SCC-Somatic cell count; MUN-Milk urea nitrogen; BW-Body weight; ADG-Average daily gain; BCS-Body condition score.
[**] NDCP-No dried citrus pulp, 0 % replacement; LDCP-Low dried citrus pulp, 33% replacement; MDCP-Medium dried citrus pulp, 66% replacement; HDCP-High dried citrus pulp, 100% replacement.
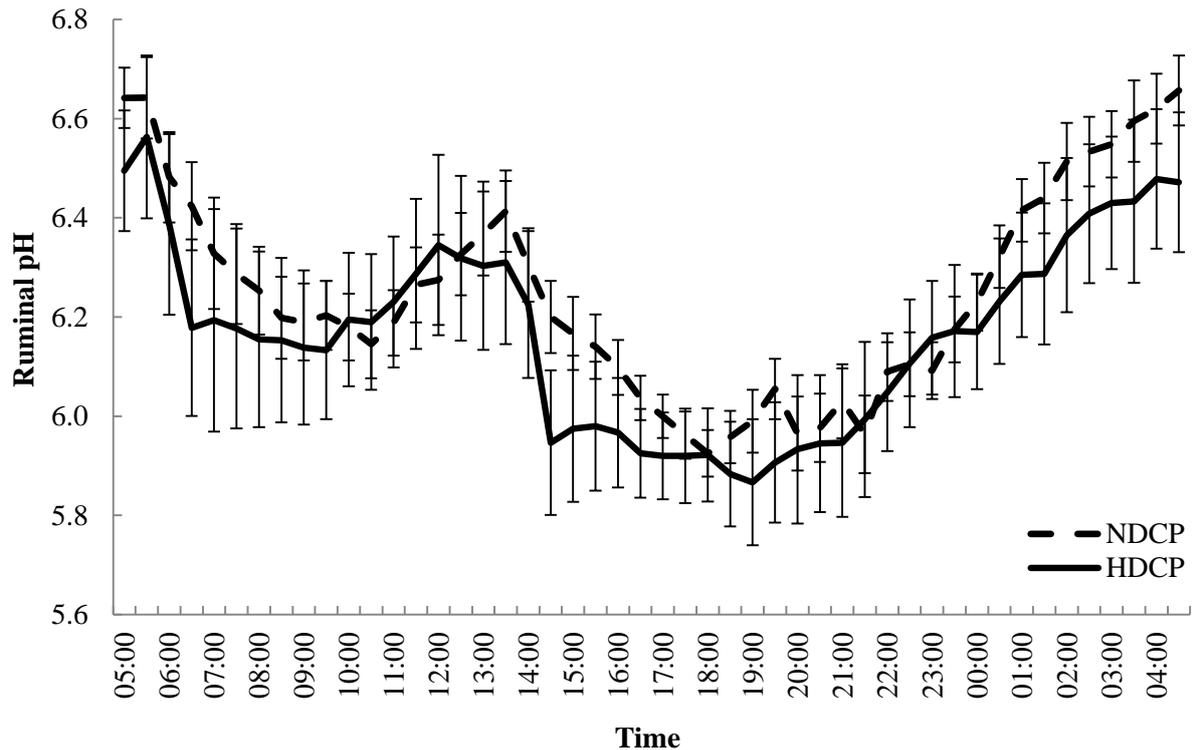
Figure 3-1 Diurnal fluctuations in ruminal pH of cows (n = 6) receiving the NDCP or HDCP concentrate supplement; error bars represent SEM, NDCP-No dried citrus pulp, 0 % replacement; HDCP-High dried citrus pulp, 100% replacement.
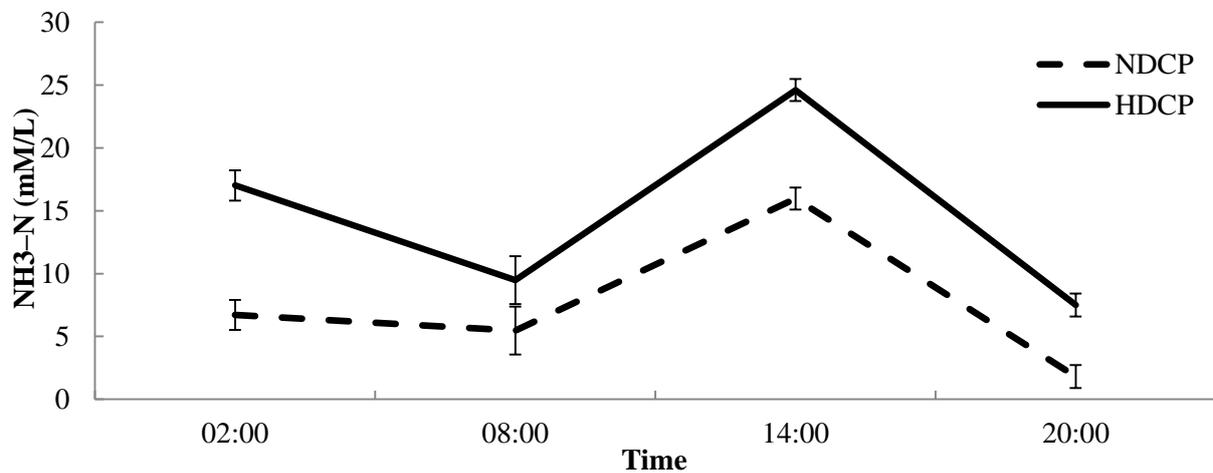
Figure 3-2 Fluctuations in ruminal NH_3-N concentration at four sampling times of cows receiving the NDCP or HDCP concentrate supplement; error bars represent SEM, NDCP-No dried citrus pulp, 0 % replacement; HDCP-High dried citrus pulp, 100% replacement.

Table 3-5 Ruminal pH parameters, fermentation products concentrations and ryegrass pasture degradability parameters (n = 6) of cows receiving the NDCP or HDCP concentrate supplement.

Parameter[*]	Treatment[**]		SEM	P-value
	NDCP	HDCP		
Mean pH	6.25	6.17	0.061	0.389
Time below (hours)				
pH 6.2	11.5	12.3	1.919	0.789
pH 6.0	5.17	6.83	2.046	0.580
pH 5.8	1.25	2.75	1.095	0.361
Total VFA (mM)	120	118	4.120	0.634
Mol/100 mol				
Acetate	62.4	62.8	0.839	0.541
Propionate	19.7	19.9	0.801	0.712
Butyrate	15.2	14.8	0.521	0.682
Valerate	1.17	1.13	0.059	0.398
Isobutyrate	0.78	0.62	0.050	0.045
Isovalerate	0.88	0.67	0.101	0.023
Acetate:Propionate	3.19	3.18	0.166	0.931
NH_3-N (mM/L)	7.49	14.7	0.885	< 0.001
Pasture degradability[***]				
DM a	20.4	20.7	0.374	0.602
b	68.4	68.4	0.746	0.984
c	0.06	0.06	0.001	0.756
NDF a	19.9	21.4	1.122	0.280
b	72.9	70.6	1.791	0.264
c	0.03	0.04	0.002	0.272

[*] VFA-Volatile fatty acids; NH3-N-Ammonia nitrogen;

[**] NDCP-No dried citrus pulp, 0 % replacement; HDCP-High dried citrus pulp, 100% replacement.

[***] Calculated with the equation: p = a+b (1-e-ct), where, a = intercept of degradation curve at t = 0; b = potential degradability of component; c = rate constant for degradation of coefficient b (Ørskov & McDonald, 1979).
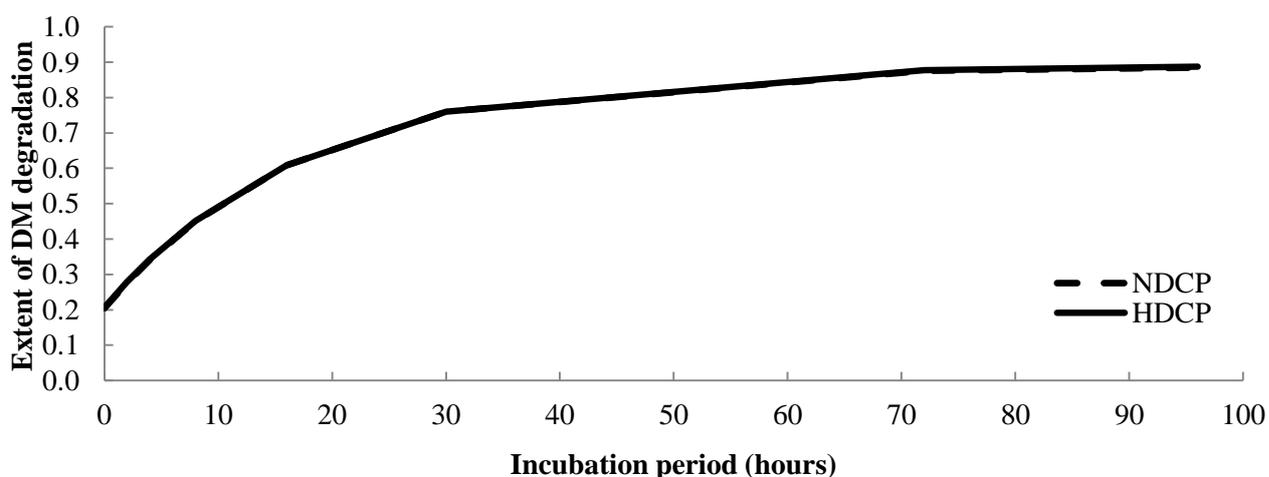
Figure 3-3 Extent of DM degradation in the rumen of cows receiving the NDCP or HDCP concentrate supplement over 96 hours of incubation (NDCP-No dried citrus pulp, 0 % replacement; HDCP-High dried citrus pulp, 100% replacement).
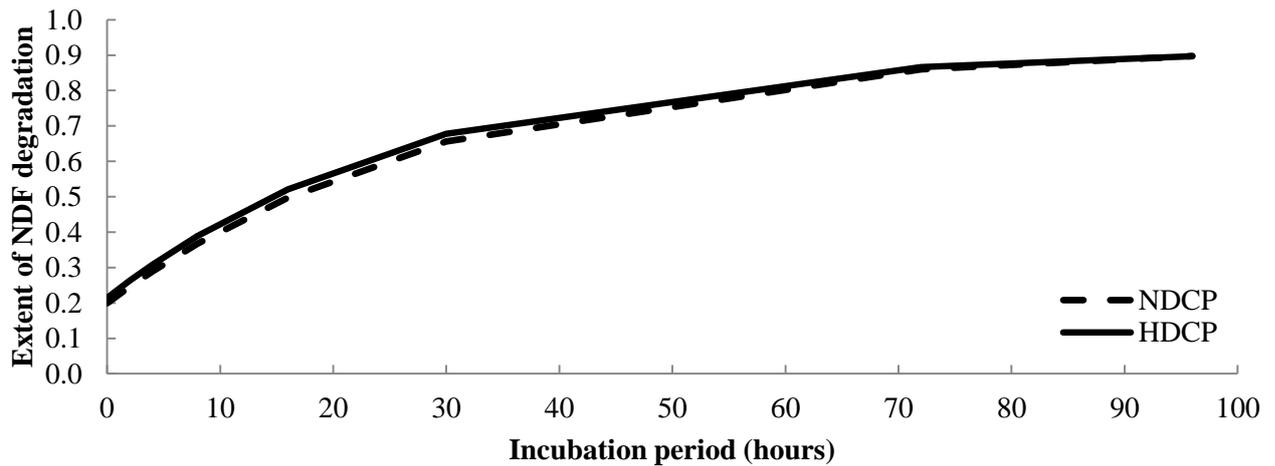
Figure 3-4 Extent of NDF degradation in the rumen of cows receiving the NDCP or HDCP concentrate supplement over 96 hours of incubation (NDCP-No dried citrus pulp, 0 % replacement; HDCP-High dried citrus pulp, 100% replacement).

3.4 Discussion

All the diets offered similar ME intakes, yet cows on the NDCP treatment yielded 2.13, 2.27 and 3.23 kg/day more milk than cows on the LDCP, MDCP and HDCP treatments, respectively. In various TMR- based trials, the level of starch was decreased by the addition of molasses or pure sucrose or by the substitution of hominy chop with citrus pulp (Leiva *et al.*, 2000; Cherney *et al.*, 2003; Broderick *et al.*, 2008); however, in each of these trials no change in milk yield was found. Alternatively it is possible that the higher NDF content of the LDCP, MDCP and HDCP concentrate supplements could limit pasture DMI due to rumen fill; however, individual pasture DMI was not measured. Furthermore, in a study by Delahoy *et al.* (2003) the partial substitution of maize with beet pulp (NDF content 295 g/kg DM), did not have an effect on pasture DMI and did not result in substitution for Holstein cows grazing Orchardgrass (*Dactylis glomerata*). Similarly, Higgs *et al.* (2013) found no effect on pasture DMI when a high NDF supplement was fed (385 g/kg DM) to Friesian cows grazing Perennial ryegrass (*Lolium perenne*). The results of these studies make the possibility of increased pasture substitution due to higher NDF content seem unlikely.

An increase in milk fat was anticipated as ground maize was replaced with DCP due to the increase in NDF content (Broderick *et al.*, 2008; Penner & Oba, 2009). However, there was no subsequent increase in ruminal pH or an increase in degradability of pasture DM and NDF. Treatment also did not affect the acetate: propionate ratio; thus no difference in milk fat content was observed. The SCC for cows on the NDCP treatment was about half of that for cows on the LDCP, MDCP and HDCP treatments; however, this is not physiologically significant as it

was well under the current legal standard of <500 x 10^3 cells/mL (Petzer *et al.*, 2017) and is indicative of good udder health. The quadratic effects on milk protein and milk lactose content for cows in the LDCP and MDCP treatments could be indicative of possible associative effects between multiple feed components (Doyle *et al.*, 2005). In a study by Higgs *et al.* (2013) a decrease in milk protein was found for cows receiving molasses as the only supplement to pasture, compared to cows receiving high maize-based concentrate supplements. This decrease in milk protein is most probably due to a lack of NFC in the diet, lowering the ruminal micro-organisms ability to utilise N from pasture (Heldt *et al.*, 1999; McCormick *et al.*, 2001). It is not known what the ruminal NH_3-N concentration in the LDCP and MDCP treatments were; however, it could be assumed that the ruminal NH_3-N would not have been high due to the efficient incorporation into MP. Treatment had no effect on BW gain, BCS change or ADG. There was, however, an improvement in BCS for cows in all treatments, and all cows gained weight. This suggests that the concentrate supplements were sufficient to maintain and slightly improve BCS of cows in early to mid-lactation.

An increase in ruminal pH and more time spent above pH 5.8 were expected, but not found. Pectin is degraded very rapidly in the rumen, but it differs from starch in the sense that it is not fermented to lactate and in this manner it does not contribute as much to the decline in ruminal pH (Strobel & Russell, 1986; Bampidis & Robinson, 2006). Pectin fermentation also ceases under low ruminal pH, and there is no cumulative effect of lowered ruminal pH (Strobel & Russell, 1986; Allen, 2001). Sugar provides less carbon for VFA production per unit of mass compared to starch (Hall & Herejk, 2001) and increases the passage rate and production of MP (Sutoh *et al.*, 1996; Ribeiro *et al.*, 2005), essentially providing less OM for fermentation. Furthermore, rumen micro-organisms are also able to convert sucrose to glycogen for short term energy storage (Hall & Weimer, 2007), temporarily reducing VFA production in the rumen (Oba, 2011) and thereby minimising the potential negative effect on ruminal pH. The higher NDF content of the HDCP concentrate supplement could also be expected to contribute to a higher ruminal pH; however, this NDF is not physically effective and thus does not help stimulate rumination and salivation.

No response on total VFA concentration was found when ground maize was replaced with DCP, which is similar to *in vivo* results reported in previous studies (Khalili & Huhtanen, 1991; Chamberlain *et al.*, 1993, Leiva *et al.*, 2000 and Sannes *et al.*, 2002). No change in the acetate and butyrate concentration as the sugar inclusion was increased corresponds with the milk fat content that was also similar between the NDCP and HDCP treatments. The propionate

concentration was also not different between the NDCP and HDCP treatments and does not provide an explanation for the decrease in milk yield when ground maize was substituted with DCP. Increased levels of sugar in the diet have been shown to decrease the acetate concentration (Khalili & Huhtanen, 1991; Chamberlain *et al.*, 1993; Broderick *et al.*, 2008), increase butyrate concentration (Khalili & Huhtanen, 1991, Chamberlain *et al.*, 1993) and also result in no change in acetate concentration or the acetate: propionate ratio (Khalili & Huhtanen, 1991; Leiva *et al.*, 2000; Oelker *et al.*, 2009). In theory, the replacement of ground maize with DCP should lead to a change in the VFA profile of the rumen; however, effects are confounding and no clear trend can be identified from previous literature. Cows in the NDCP treatment had a higher concentration of isobutyrate and isovalerate than cows in the HDCP treatment, similar to Khalili & Huhtanen (1991). Branched chain volatile fatty acids (BCVFA) are essential for the effective functioning of cellulolytic bacteria and the synthesis of microbial protein, specifically the amino acids valine, isoleucine, leucine and proline (Cummins & Papas, 1985; Andries *et al.*, 1987). Broderick *et al.* (2008) found a decrease in total BCVFA concentration as the level of sugar in the diet increased.

The concentration of NH_3-N is dependent on how efficiently ruminal micro-organisms are able to utilise the N from NH_3-N for MP production and this is determined by the availability of the energy source (Bach *et al.*, 1999; Heldt *et al.*, 1999; Higgs *et al.*, 2013). The NDCP concentrate supplement had a higher NFC content than the HDCP concentrate supplement, thus the ability of micro-organisms of cows on the HDCP treatment to utilise the N from ruminal NH_3-N could have been limited by the availability of NFC. There was no difference in milk protein content between the NDCP and HDCP concentrate supplements, thus there was no indication of improved utilisation of NH_3-N by ruminal micro-organisms. The CP content of the HDCP concentrate supplement was slightly higher than that of the NDCP concentrate supplement and could possibly have contributed to the higher ruminal NH_3-N concentration. Cows in the HDCP treatment had a tendency towards higher MUN content, which could be indicative of an oversupply of NH_3-N in the rumen (Jonker *et al.*, 1998). However, the MUN content of all four treatments was within the normal acceptable range of 8-12 mg/dL (Kohn, 2007), and the trend does not hold much biological significance. The daily ruminal NH_3-N concentration was higher for cows in the HDCP treatment; however, these values fall within the accepted range of 8.7-32.2 mg/dL (Bargo *et al.*, 2003). It could be postulated that the decrease in milk yield as the level of DCP inclusion increased, which corresponded to an increase in soybean oilcake inclusion, could be due to an oversupply of protein, with energy

being diverted to deal with the excess protein supply instead of being used for milk production (Broderick, 2003). The CP content of the four experimental diets ranged from 117-121 g/kg DM; however, the protein fractions (rumen undegradable protein, rumen degradable protein and amino acids) were not investigated and were beyond the scope of this study. There are many complex interrelationships that play a role here and it was not a focus point of the study.

An improvement in pasture degradability was expected for cows in the HDCP treatment; however, no improvement in ruminal pH was found, and as a result there was no improvement in pasture degradability. A higher mean ruminal pH with fewer and shorter dips below pH 6.0 would promote cellulolytic bacteria activity, increasing the degradability of pasture (Calsamiglia *et al.*, 2002; Jacobs, 2014). Earlier results with regard to the effect of increasing sugar and pectin content on NDF degradability of roughages have been varying, with some studies finding no improvement (Penner & Oba, 2009) and others finding a linear and quadratic effect (Broderick & Radloff, 2004; Broderick *et al.*, 2008). Previous *in vitro* studies found that the use of molasses as a substrate increased the DM degradability of ryegrass pasture; however, ryegrass pasture NDF degradability remained unchanged (Malan, 2009) and the use of sucrose and pectin as a substrate led to a linear and quadratic increase in NDF degradation at 24 hours of incubation, respectively (Holtshausen, 2004). Improved pasture DM and NDF degradability could potentially increase pasture DM intake, promoting a more stable rumen environment and increasing production. The lack of response in pasture DM and NDF degradability should be investigated further.

3.5 Conclusion

Replacing ground maize with DCP caused a decrease in milk yield and 4% FCM yield. Milk protein and milk lactose content were not affected. Rumen health and activity were maintained and no improvement was seen. The use of DCP as a substitute for ground maize in a concentrate supplement fed to Jersey cows grazing ryegrass pasture should only be considered if it is available at a lower cost than maize, making up for the decrease in income over feed cost due to a possible decrease in milk production.

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Chapter 4: The effect of replacing maize with dried apple pomace in the concentrate on performance of Jersey cows grazing kikuyu pasture

Abstract

Dried apple pomace (DAP) is a by-product of the apple juice industry. It has a comparable ME and CP content maize but has a higher NDF content. It has been used as a substitute to maize with success in various TMR systems, offering an affordable alternative to maize. The aim of the study was to determine whether the replacement of ground maize with DAP would be beneficial to milk production and rumen health of Jersey cows grazing kikuyu pasture. Seventy two lactating Jersey cows were blocked according to milk yield, days in milk and lactation number. Cows within blocks were randomly allocated to one of four treatments. Treatments were: 0% DAP inclusion (AP 0), 25% DAP inclusion (AP 25), 50% DAP inclusion (AP 50) and 75% DAP inclusion (AP 75). Cows received 6 kg as is/day of the allocated concentrate in the milking parlour. Cows of all four treatments strip grazed kikuyu-ryegrass pasture, as one group, over an area of 8.6 ha. A rising plate meter was used to estimate pasture DM yield pre- and post-grazing and manage the allocation of pasture. Pasture samples and feed samples were collected on a weekly basis, dried at 60°C for 72 hours and pooled every two weeks. An additional eight ruminally cannulated cows were used and were subjected to a four period crossover design with a 14 day adaptation period between treatments. Rumen pH, pasture DM and NDF degradability and VFA profile were determined. Treatment did not have an effect on milk yield; however, there was a linear decrease in 4% FCM and fat yield as the level of DAP inclusion in the diet increased. Cows receiving the AP 0 concentrate supplement yielded 0.9 and 1.2 kg more 4% FCM than cows on the AP 50 and AP 75 concentrate supplements (P <0.001), respectively. Treatment had no effect on milk composition, except for the lactose content which was lower for cows receiving the AP 0 concentrate supplement (P <0.001). Mean rumen pH was lower for cows receiving the AP 75 concentrate supplement (P <0.001); however, treatment did not affect the VFA profile or pasture DM and NDF degradability. It was concluded that the replacement of ground maize with DAP for cows grazing kikuyu pasture is a viable option; however, the possible decrease in 4% FCM yield should be taken into consideration.

Key words: fruit waste, non-forage fibre sources, pasture degradability, pasture supplementation.

4.1 Introduction

In the southern Cape area of South Africa kikuyu (*Pennisetum clandestinum*) is a main pasture base, especially during summer (Botha *et al.*, 2008). This is despite its nutritional inadequacies, which include low readily digestible non-structural carbohydrates (NSC) and high NDF content (Marais, 2001). To compensate for the low nutritive value and low digestibility of kikuyu pasture and maintain milk production, additional supplementation is commonly provided to lactating cows. Most often a highly digestible concentrate supplement, high in ME and CP, is fed to cows in the milking parlour. Such a concentrate usually contains high levels of ground maize. In previous studies it has been shown that supplementing cows on kikuyu pasture with a high fibre concentrate supplement compared to a low fibre concentrate supplement yielded comparable milk production and composition (Cawood, 2016; Van Wyngaard & Meeske, 2017). High fibre feed sources, such as fruit waste, are often more cost effective compared to low fibre sources, such as maize. Furthermore, low fibre, maize based concentrate supplements are high in starch, which has been shown to be detrimental to the ruminal pH and the cellulolytic bacteria population (Berzaghi *et al.*, 1996; Calsamiglia *et al.*, 2010; Poulsen *et al.*, 2012). Dried apple pomace (DAP), a by-product of the apple juicing industry, is a product that is high in digestible NDF (483-612 g/kg DM) and comparable to maize in ME (7.8-11.6 MJ/ kg DM) and low in CP content (77-80 g/kg DM; National Research Council, 2001; Edwards & Parker, 1995; Mirzaei-Aghsaghali *et al.*, 2011). The chemical composition of DAP varies widely depending on the processing methods applied as well as the specific apple variety and how it was managed post-harvest (Kennedy *et al.*, 1999). Apple pomace (dry or wet ensiled) has been used with success in TMR diets fed to lactating dairy cows (Edwards & Parker, 1995); however, little information is available on DAP fed to cows grazing pasture as the main roughage source. The aim of the study was to determine the effect of replacing ground maize with DAP on milk yield and rumen health of Jersey cows grazing kikuyu pasture.

4.2 Materials and methods

The trial was conducted during summer and early autumn of 2016 (March - May) on the Outeniqua Research Farm, situated in the Western Cape province of South Africa (22º 25' 16" E and 33º 58' 38" S). The mean minimum and maximum temperatures and total rainfall during the study period were 11.9°C, 23.2°C and 108.2 mm, respectively (ARC, 2016). The study area

(8.6 ha) consisted of a permanently maintained kikuyu pasture (*Pennisetum clandestinum*) and was characterised by a Witfontein soil form (Swanepoel *et al.* 2013). Perennial ryegrass (*Lolium perenne*, cv. Bealey) was seeded into the kikuyu base at a rate of 20 kg/ha using an Aitcheson seeder during April of the previous year. Kikuyu was the predominant pasture available to cows (42%) with the rest of the pasture consisting of 26% perennial ryegrass, 7% legume and 26% other grasses. Individual paddocks were fertilised with 42 kg N/ha using limestone ammonium nitrate (280g N/kg) post-grazing.

Four different treatments were applied in this study, Table 4-1. The treatments where identified by the level of DAP included in the concentrate supplement and were: 0% DAP (AP 0), 25% DAP (AP 25), 50% DAP (AP 50) and 75% DAP (AP 75). The study involved three main components, namely a production study, a pasture DMI study and a rumen metabolism study. All study components ran simultaneously, in conjunction with each other. Ethical clearance was granted by the Research Ethics Committee of Stellenbosch University, South Africa (SU-ACUD15-00094).

In the production study, 72 lactating Jersey cows were blocked according to days in milk (mean ± SD; 114 ± 46.2 d), lactation number (3.8 ± 1.45) and mean milk yield over the previous 3 weeks (16.1 ± 2.11 kg). Cows within blocks were then randomly assigned to treatments in a complete randomised block design. All cows received 3 kg as is of the respective treatment diet at each milking (05:30 and 13:30), for a total daily intake of 6 kg/cow.. A rising plate meter (RPM), along with the linear regression equation: $Y = 76.8 * H\text{-}287$, where Y = DM yield and H = RPM reading, was used to estimate the DM yield of pasture (van der Colf, 2011). Pasture was then strip grazed to ensure an estimated daily pasture DMI of 10 kg DM/cow and water was available *ad libitum* in the paddocks. Milk yield was automatically recorded at each milking via the Dairy Master milking system. Milk samples were collected once every two weeks during the morning and afternoon milking sessions and a composite sample was then preserved with Bronopol. A 24 mL composite sample was collected, with 1 mL = 1 hour, taking the time between milking into consideration. Samples were analysed for fat, protein, lactose, MUN and SCC (FOSS CombiFoss[TM] FT+; FOSS, Foss Allè 1, DK–3400 Hillerød, Denmark). The net energy for lactation of cows of the four different treatment groups was calculated as: NE_L (Mcal/kg) = 0.0929 x Fat% + 0.0547 x Protein% + 0.0395 x Lactose% (National Research Council, 2001). The 4% fat corrected milk (FCM) yield was determined using the Gaines formula (Gaines, 1928), where 4% FCM = (0.4*kg milk) + (15*kg fat) to correct milk yield to a constant fat basis. Cows were weighed on two consecutive days, post-afternoon milking, at

the commencement and completion of the trial (Tru-Test EziWeigh v 1.0 scale; Auckland, NZ). Mean weights were used to reduce any disparity which could arise from variation in pasture DMI, urination and defecation. The BCS of cows were recorded on the first and last days of the trial. The five point scale as described by Wildman *et al.* (1982) and Edmonson *et al.* (1989) was used, where a score of one indicates an extremely thin cow and a score of five indicates an extremely fat cow. Average daily gain (ADG) was calculated as the weight difference obtained at the start and end of the trial period of 51 days.

Pasture samples were collected at a height of 30 mm, once a week, one day prior to grazing. Pasture samples were then dried at 60˚C for 72 hours to determine pasture DM. Additional samples were collected for fractionation to determine the botanical composition. This was done on three occasions, evenly spread out over the duration of the trial. Pasture samples were also cut once a week for the development of a regression equation, towards improving the accuracy for estimating pasture DM availability using the RPM. Feed samples were collected once a week, dried at 60˚C for 72 hours and pooled over two weeks. All pasture and feed samples were analysed for organic matter (OM; AOAC, 2002; method 942.05), crude protein (CP; AOAC, 2002; method 990.03; using the Leco N analyser, model FP 528) and neutral detergent fibre (NDF; Robertson & van Soest, 1981; using the Ankom fibre analysis system with heat stable α-amylase, followed by incineration of the residue) and acid detergent fibre (ADFom; Robertson & van Soest, 1981; using the Ankom Fibre Analyzer, ANKOM Technology, Fairport, NY, USA). Samples were also analysed for neutral detergent insoluble nitrogen (NDIN; NDF procedure, residue analysed for CP), ether extract (EE; AOAC, 2002; method 920.39), gross energy (GE; MC1000 Modular Calorimeter, Energy Instrumentation, Sandton, South Africa, 2146), *in vitro* organic matter digestibility (IVOMD; Buys *et al.*, 1996), starch (AOAC, 2002; method 996.11), sugar (AOAC, 1998; Method 982.14), Ca (ALASA, 1998; Method 6.1.1), P (ALASA, 1998; Method 6.1.1), K (ALASA, 1998; Method 6.1.1) and Mg (ALASA, 1998; Method 6.1.1). Metabolisable energy (ME) was calculated as: ME (MJ/kg DM) = GE x IVOMD x 0.82 (Robinson *et al.*, 2004) and non-fibrous carbohydrates (NFC) content as: NFC (g/kg DM) = 100-(NDF+CP+EE+Ash) (National Research Council, 2001).

Titanium dioxide was used to estimate pasture DMI. Ten cows in each treatment were dosed orally with 3g of TiO_2 twice a day for ten days (6 g TiO_2/day). One additional cow per treatment was included for background analysis, these cows were not dosed. Voluntary and grab faecal samples were collected twice a day on days 6 to 10 of the dosing period (Glindemann *et al.*, 2009). Faecal samples were then dried at 55˚C for 72 hours (De Souza *et al.*, 2015). After

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drying samples were pooled per cow and then ground through a 1mm sieve (Glindemann *et al.*, 2009). Faecal samples of dosed and background cows, pasture samples and feed samples were then analysed for TiO_2 content by the method of Myers *et al.* (2004) to determine faecal excretion. Total faecal excretion was calculated with the equation: Total faecal excretion (g/day) = Daily dose of TiO_2 (g/day)/ TiO_2 concentration in faeces (g/day) (de Souza *et al.*, 2015). The indigestible NDF (iNDF) of faeces, pasture and feed samples were determined using *in vitro* incubation for 240 hours, under continuous CO_2 infusion according to Goering & van Soest (1970), followed by NDF analysis of the residue (Goering & van Soest, 1970). Finally, pasture DMI was determined by the following equation: Pasture DM intake (kg/day) = [{Total faecal excretion (kg/day) x iNDF of faeces (kg/kg)}-iNDF intake from concentrate (kg/day)]/ iNDF of forage (kg/kg) (Cabral *et al.*, 2014).

Eight ruminally cannulated cows were used in the rumen metabolism study. Two cannulated cows were randomly allocated to each treatment and subjected to a four period cross over design. Cows of the rumen study grazed alongside cows of the production study and were subjected to the same experimental procedures with respect to feeding and milking. A fourteen day adaptation period was applied between each rumen sampling period. Rumen fluid samples were collected with a customised hand pump inserted into the rumen via a 5 mm hole in the cannula plug and were collected at eight-hour time intervals; 06:00, 14:00 and 22:00. After collection, samples were filtered through a double layer of cheesecloth after which an aliquot sample was frozen in an airtight container and immediately frozen for later analysis of NH_3-N (Broderick & Kang, 1980) and VFA (Filípek & Dvořák, 2009).

TruTrack pH Data Loggers (Model pH-HR mark 4, Intech Instruments LTD, New Zealand, www.intech.co.nz) were used for the recording of rumen pH values over a 96 hour period. Loggers were carefully calibrated with two buffer solutions (pH 4 and 9) and tested with a buffer of pH 7. Once calibration was completed, loggers were placed in a standard solution of pH 7 for 12 hours. Loggers were then inserted into the rumen for a 96 hour logging period. Once logging was complete loggers were removed from the rumen and placed in a standard solution of pH 7 for 6 hours. Any drift in pH recorded was used to correct the pH data.

Pasture dry matter degradability (DMD) and pasture NDF degradability (NDFD) were determined through the use of *in sacco* nylon bags (Bar Diamond Inc, PO Box 60, Parma, Idaho, USA), with a mean pore size of 53 µm and inner size of 10 cm x 20 cm, containing dried and cut (5 mm) kikuyu pasture samples. A sample size to bag surface area ratio of 12.5 mg/cm^2 was obtained, which is sufficiently low to ensure efficient degradability (Vanzant *et al.*, 1998).

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The bags were incubated in the rumen for 6, 18 and 30 hours using the method as described by Cruywagen (2006). After incubation, all bags were washed in a twin tub washing machine for 3 minutes and dried at 60˚C for 72 hours. Bag residues were analysed for DM (AOAC, 2002; method 934.01) and aNDFom concentration. Pasture DMD and NDFD was determined with the rate calculator of van Amburgh *et al.* (2003).

Milk yield, milk composition, body weight (BW), body condition score (BCS) and pasture DMI data were subjected to a mixed model procedure, using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Polynomial contrasts were also included to test for linear, quadratic and cubic effects of replacing ground maize with DAP at increasing levels. Covariance was not included due to the blocking of cows. Volatile fatty acid and NH_3-N data were analysed using a mixed model procedure over time, with polynomial contrasts also included. The *in sacco* Dacron bag study data were subjected to a main effects ANOVA and Polynomial contrasts. The ruminal pH data were subjected to a repeated measures ANOVA and polynomial contrasts. Tukey's test was used to compare the treatment means at a 5% significance level. The null hypothesis was: Ho: $\mu_1 = \mu_2 = \mu_3 = \mu_a$. The null hypothesis was rejected where $P < 0.05$. Least squares means were used to calculate a pooled standard error of treatment means. Shapiro-Wilk tests were used to test for normality (Shapiro and Wilk, 1965).

4.3 Results

The four different concentrate supplements fed in the trial contained varying levels of DAP and ground maize to generate concentrate supplements ranging from high sugar and low starch to low sugar and high starch (Table 4-1). The starch level ranged from 107 g/kg DM in the AP 75 concentrate supplement to 656 g/kg DM in the AP 0 concentrate supplement, whereas the total sugar content ranged from 149 g/kg DM in the AP 75 concentrate supplement to 31 g/kg DM in the AP 0 concentrate supplement. The CP content of the four concentrate supplements were comparable ranging between 135 g/kg DM to 141 g/kg DM. The increase in NDF and ADF content as the level of AP inclusion increased is also notable, with the NDF content increasing from 164 g/kg DM to 317 g/kg and the ADF content increasing from 36.5 g/kg DM to 235 g/kg DM in the AP 0 and AP 75 concentrate supplements, respectively. The ME content of the four concentrate supplements were also similar, ranging between 11.4 and 11.8 MJ/kg DM.

Treatment did not have an effect on milk yield; however, there was a linear decrease in 4% FCM and fat yield as the level of AP inclusion increased in the diet (Table 4-2). Cows receiving

the AP 0 concentrate supplement yielded 0.9 and 1.2 kg more 4% FCM than cows on both the AP 50 and AP 75 concentrate supplements (P <0.001), respectively. Cows receiving the AP 0 and AP 25 concentrate supplements had a higher fat yield than cows receiving the AP 50 and AP 75 concentrate supplements (P = 0.002). Treatment had no effect on milk composition, except for the lactose content which was lower for cows receiving the AP 0 concentrate supplement (P <0.001). There were no differences between the BW of cows per treatment prior to the start of the trial; however, there was a quadratic trend towards higher BW before for cows in the AP 0 and AP 75 treatments. Treatment did not affect BW change, ADG or BCS change.

Mean pre- and post-grazing pasture heights of 34 and 11.8 respectively, were obtained using the RPM (Table 4-3). With the use of the RPM for pasture allocation, mean daily pasture DMI for the whole group was estimated as 10.5 kg DM/cow. This deemed to be an over-estimation when compared to the pasture DMI values obtained with the use of the TiO_2 maker method (Table 4-4), which estimated an average pasture DMI of 8.12 kg/d. Treatment did not affect faecal excretion, pasture DMI, total DMI or DMI as % BW. Treatment did have an effect on the NDF intake in relation to BW, with cows receiving the AP 75 concentrate supplement consuming more NDF compared to cows on either of the AP 0, AP 25 and AP 50 concentrate supplements in relation to BW (P = 0.004).

The total daily ME intake and the net energy in milk did not differ between treatments and ranged between 125 and 135 MJ/cow (Table 4-5). The total amount of energy secreted through the milk was highest for cows receiving the AP 0 concentrate supplement and lowest for cows receiving the AP 75 concentrate supplement (P = 0.001); however, when ME intake was converted to energy in milk, treatment did not have any effect. The amount of milk produced from every kg feed received did not differ between treatments; however, there was a cubic trend where cows receiving the AP 50 concentrate supplement produced 1.08 kg milk for every 1 kg DM feed consumed and cows receiving the AP 75 concentrate supplement produced only 0.96 kg milk per 1 kg DM feed consumed; however, this may possibly be a random treatment effect.

Table 4-1 Ingredient and chemical composition of the four concentrate supplements, DAP and pasture used in the study (n = 3).

Parameter[*]	Treatment[**]				DAP	Pasture[***]
	AP 0	AP 25	AP 50	AP 75		
Ingredient (g/kg)						
Ground maize	750	500	250	0		

DAP	0	250	500	750		
Soybean oilcake	125	125	125	125		
Wheat bran	70	47	24	0		
Molasses (liquid)	20	40	60	81		
Feed lime	21	19	17	16		
Salt	6.0	6.0	6.0	6.0		
Urea	3.0	6.0	8.0	11		
Premix[****]	1.0	1.0	1.0	1.0		
MgO	3.0	3.0	3.0	3.0		
Mono-CaP	1.0	3.0	6.0	7.0		
Chemical (g/kg DM)						
DM	900	915	917	917	921	158
OM	946	928	936	939	981	892
CP	135	142	141	141	67.4	196
EE	18.1	30.7	30.8	28.4	37.1	29.0
NFC	627	586	526	452	435	216
Total sugar	31.0	68.8	115	148	115	179
Fructose	4.60	21.1	46.0	72.9	54.3	17.2
Glucose	3.60	14.7	28.5	37.8	52.5	7.2
Sucrose	22.8	33.0	40.5	37.8	8.20	14.5
Starch	656	550	328	107	91.0	41.2
NDF	164	169	238	317	442	450
ADF	36.5	71.4	153	235	354	222
ADL	6.1	23.1	46.2	68.5	134	28.7
IVOMD	885	862	833	784	785	652
GE (MJ/kg DM)	15.9	16.2	16.9	17.3	20.1	14.9
ME (MJ/kg DM)	11.8	11.7	11.8	11.4	13.2	8.17
Ca	7.88	11.8	10.2	9.96	1.43	3.35
P	4.46	5.77	4.45	4.45	1.54	4.52
Ca: P	1.77	2.04	2.30	2.24	0.93	0.75
Mg	3.46	4.55	4.08	3.79	0.99	4.06
K	8.59	10.4	11.0	12.5	6.50	41.4

[*] DAP-Dried apple pomace; DM-Dry matter; OM-Organic matter; CP-Crude protein; EE-Ether extract; NFC-Non-fibrous carbohydrates; NDF-Neutral detergent fibre; ADF-Acid detergent fibre; IVOMD-In vitro organic matter degradability; GE-Gross energy; ME-Metabolisable energy.

[**] AP 0-0% DAP inclusion; AP 25-25% DAP inclusion; AP 50-50% DAP inclusion; AP 75-75% DAP inclusion.

[***] Pasture-Kikuyu pasture (*Pennisetum clandestinum*).

[****] Premix-4 mg/kg Cu; 10 mg/kg Mn; 20 mg/kg Zn; 0.34 mg/kg I; 0.2 mg/kg Co; 0.06 mg/kg Se; 6 x 106 IU vitamin A; 1 x 106 IU vitamin D3; 8 x 103 IU vitamin E (Supplier: Cape Feed and Grain, George East, 6539, South Africa).

The mean ruminal pH measured over a 24 hour period showed a linear decrease from pH 6.23 for cows receiving the AP 0 concentrate supplement to pH 6.03 for cows receiving the AP 75 concentrate supplement ($P < 0.001$), Table 4-6. Treatment did not affect the diurnal ruminal pH (Figure 4-1). Treatment did not have an effect on the time spent below pH 5.8, 6.0 or 6.2

or on the VFA concentration. Treatment did not affect the acetate to propionate ratio or the NH₃-N concertation in the rumen. The extent of pasture DMD and NDFD and the rate of pasture degradation were determined at 6, 18 and 30 hours of incubation (Table 4-7). Treatment did not have an effect on any of the degradability parameters. There was a cubic trend for DMD at 30 hours of incubation; however, that may be due to a random occurrence. Furthermore, there was a linear tendency for a lower rate of degradation for cows receiving the AP 50 concentrate supplement; however, when the values are considered they do not follow a linear pattern.

Table 4-2 Mean milk yield, milk composition and BW and BCS change of cows receiving one of four concentrate supplements (n = 18).

Parameter[*]	Treatment[**]				SEM	P-value		
	AP 0	AP 25	AP 50	AP 75		Linear	Quadratic	Cubic
Production (kg/cow)								
Milk yield	13.7	13.3	13.5	12.8	0.39	0.059	0.478	0.267
4% FCM yield	16.5[a]	16.3[ab]	15.6[bc]	15.3[c]	0.36	<0.001	0.804	0.438
Fat yield	0.73[a]	0.73[a]	0.68[b]	0.68[b]	0.02	0.002	0.927	0.158
Protein yield	0.52	0.51	0.51	0.50	0.01	0.058	0.906	0.579
Milk composition								
Fat (g/kg)	53.9	55.3	51.5	53.6	2.20	0.493	0.809	0.114
Protein (g/kg)	38.3	38.5	38.0	39.2	1.10	0.564	0.503	0.527
Lactose (g/kg)	44.1[a]	45.0[b]	45.7[b]	45.5[b]	0.40	<0.001	0.047	0.675
SCC (x 10³ cells/mL)	185	199	128	174	33.5	0.463	0.611	0.161
MUN (mg/dL)	12.2	12.2	12.2	12.8	0.43	0.199	0.361	0.561
BW (kg)								
Before	405	388	395	407	9.22	0.629	0.033	0.533
Change	11.5	10.4	8.33	2.64	4.97	0.074	0.513	0.873
ADG	0.26	0.24	0.19	0.06	0.11	0.073	0.510	0.878
BCS (Scale 1-5)								
Before	2.21	2.11	2.14	2.18	0.06	0.757	0.088	0.536
Change	0.14	0.12	0.10	0.14	0.06	0.901	0.517	0.709

[a,b] Difference in superscript indicates significance at P < 0.05.
[*] FCM-Fat corrected milk; SCC-Somatic cell count; MUN-Milk urea nitrogen; BW-Body weight; ADG-Average daily gain; BCS-Body condition score.
[**] AP 0-0% Dried apple pomace (DAP) inclusion; AP 25-25% DAP inclusion; AP 50-50% DAP inclusion; AP 75-75% DAP inclusion.

Table 4-3 Pre- and post-grazing RPM height (mean ± SD), pasture yields, pasture allowances and pasture DM intake determined using the seasonal linear regression.

Parameter[*]	Pasture values
Pre-grazing	
RPM height	34 ± 4.1
Pasture yield (kg DM/ha)[**]	2076 ± 280
Daily pasture allowance (kg DM/cow)	12.2 ± 3.03

Post-grazing	
RPM height	11.8 ± 0.85
Pasture yield (kg DM/ha)	527 ± 58.8
Estimated daily pasture DM intake (kg DM/cow)	10.5 ± 2.27

[*] RPM-Rising plate meter; DM-Dry matter.

[**] $Y = 68.8 * H - 286$, where Y = DM yield and H = RPM reading (R^2=0.82).

Table 4-4 Pasture DMI in relation to cow BW determined using TiO_2 as an internal marker (n = 10)

Parameter[*]	Treatment[**]				SEM	P-value		
	AP 0	AP 25	AP 50	AP 75		Linear	Quadratic	Cubic
Faecal excretion (kg/d)	3.56	3.57	3.48	3.69	0.13	0.608	0.463	0.526
Pasture DMI (kg/d)	8.73	8.38	7.41	7.98	0.41	0.088	0.268	0.251
Total DMI (kg/d)	14.2	13.9	12.9	13.5	0.41	0.106	0.330	0.228
BW (kg)	412	410	396	395	9.89	0.132	0.956	0.582
DMI as % BW (kg DM)	3.43	3.28	3.27	3.42	0.10	0.925	0.155	0.958
NDF intake as % BW (kg DM)	1.17[b]	1.11[b]	1.18[b]	1.35[a]	0.04	0.004	0.012	0.906

[a,b] Difference in superscript indicates significance at P < 0.05.

[*] DMI-Dry matter intake; BW-Body weight; NDF-Neutral detergent fibre.

[**] AP 0-0% Dried apple pomace (DAP) inclusion; AP 25-25% DAP inclusion; AP 50-50% DAP inclusion; AP 75-75% DAP inclusion.

Table 4-5 Mean daily energy intake and excretion (milk) and conversion of energy into milk of cows receiving one of four concentrate supplements (n = 10).

Parameter[*]	Treatment[**]				SEM	P-value		
	AP 0	AP 25	AP 50	AP 75		Linear	Quadratic	Cubic
Total ME intake (MJ/cow)	135	133	125	128	3.45	0.054	0.486	0.329
NE_L (MJ/L) in milk	3.61	3.76	3.57	3.72	0.10	0.718	0.991	0.078
Energy in milk (MJ/day)	51.9[a]	50.1[ab]	48.8[b]	47.7[b]	1.01	0.001	0.689	0.903
Conversion of ME intake to milk energy (%)	38.5	37.9	39.1	37.7	1.18	0.819	0.673	0.386
Kg milk/kg feed	1.02	0.98	1.08	0.96	0.05	0.651	0.355	0.038

[a,b] Difference in superscript indicates significance at P < 0.05.

[*] NE_L-Net energy for lactation (Mcal/kg) = 0.0929 x Fat% + 0.0547 x Protein% + 0.0395 x Lactose% (National Research Council, 2001); 1 Mcal = 4.184 MJ.

[**] AP 0-0% Dried apple pomace (DAP) inclusion; AP 25-25% DAP inclusion; AP 50-50% DAP inclusion; AP 75-75% DAP inclusion.

Table 4-6 Mean ruminal pH and time that the rumen spent below pH 6.2, 6.0 and 5.8, individual VFA concentrations and mean NH$_3$-N concentration of cows receiving one of four concentrate supplements.

Parameter[*]	Treatment[**]				SEM	P-value		
	AP 0	AP 25	AP 50	AP 75		Linear	Quadratic	Cubic
Mean pH	6.23[a]	6.21[a]	6.13[b]	6.03[c]	0.04	<0.001	0.040	0.590
Time below (hrs)								
pH 6.2	9.85	11.1	9.51	14.3	2.60	0.345	0.538	0.473
pH 6.0	5.38	7.20	5.80	10.0	2.67	0.329	0.684	0.502
pH 5.8	3.71	3.92	5.84	7.58	2.69	0.296	0.797	0.885
VFA (mM/L)								
Acetate	66.3	58.9	55.7	62.1	4.85	0.471	0.169	0.810
Propionate	20.0	16.9	15.7	17.6	1.69	0.273	0.147	0.874
Butyrate	13.9	11.5	11.7	12.7	1.10	0.505	0.125	0.713
Iso-butyrate	1.63	1.30	1.45	1.49	0.12	0.597	0.127	0.261
Iso-valerate	0.95	0.73	0.84	0.86	0.08	0.636	0.135	0.204
Valerate	0.53	0.42	0.43	0.48	0.04	0.466	0.066	0.612
Acetate:Propionate	3.32	3.49	3.66	3.62	0.13	0.059	0.410	0.688
NH$_3$-N (mM/L)	14.2	14.8	14.7	15.6	0.78	0.252	0.799	0.581

[a,b] Difference in superscript indicates significance at P < 0.05.

[*] VFA-Volatile fatty acids; NH$_3$-N-Ammonia nitrogen

[**] AP 0-0% Dried apple pomace (DAP) inclusion; AP 25-25% DAP inclusion; AP 50-50% DAP inclusion; AP 75-75% DAP inclusion.
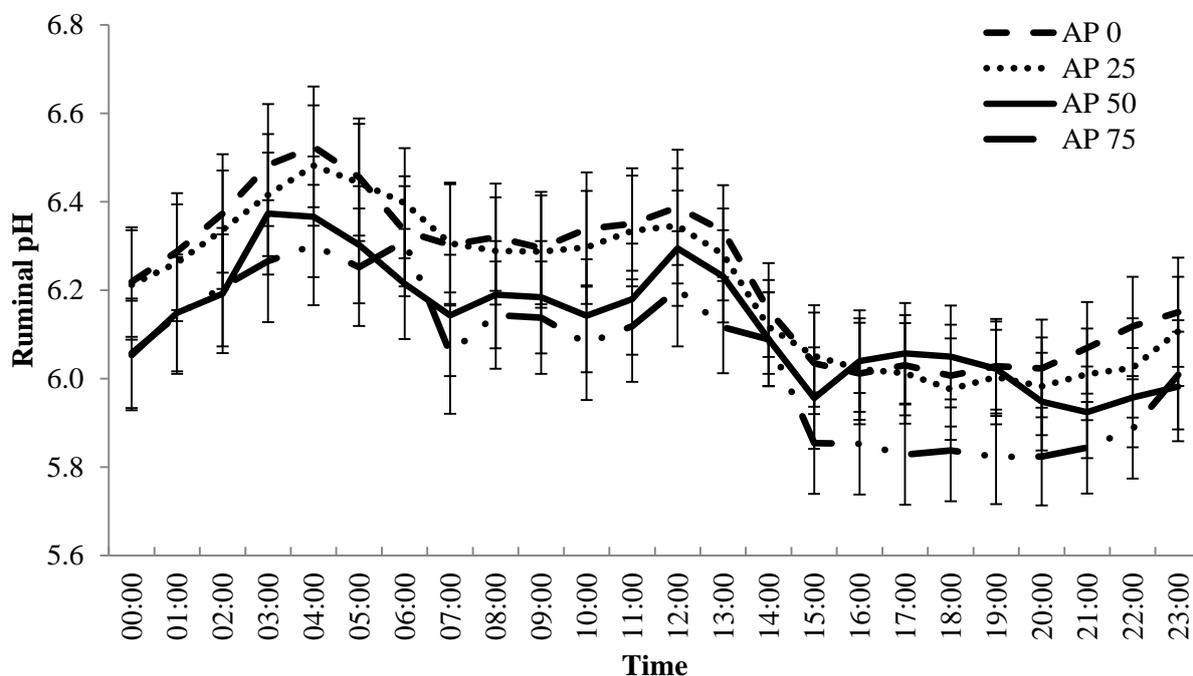
Figure 4-1 Diurnal fluctuations in ruminal pH of cows (n = 8) receiving one of four concentrate supplements; error bars represent SEM, AP 0-0% Dried apple pomace (DAP) inclusion, AP 0-0% Dried apple pomace (DAP) inclusion; AP 25-25% DAP inclusion; AP 50-50% DAP inclusion; AP 75-75% DAP inclusion.

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Table 4-7 *In vivo* Kikuyu pasture degradability parameters (n = 8) calculated with the rate calculator of van Amburgh *et al.* (2003) using *in sacco* Dacron bags at 6, 18 and 30 hours of incubation.

Parameter[*]	Treatment[**]				SEM	P-value		
	AP 0	AP 25	AP 50	AP 75		Linear	Quadratic	Cubic
DMD (%)								
6 h	29.1	29.2	28.5	29.1	0.44	0.733	0.545	0.277
18 h	42.9	42.4	40.1	43.0	1.01	0.686	0.121	0.141
30 h	58.7	60.3	55.9	58.7	1.15	0.356	0.601	0.013
NDFD (%)								
6 h	13.4	14.1	12.7	15.1	0.71	0.246	0.258	0.080
18 h	30.4	30.4	26.8	31.5	1.63	0.962	0.140	0.102
30 h	54.8	53.7	49.5	51.8	1.70	0.096	0.317	0.220
kd (%/h)								
6 h	2.87	3.05	2.73	3.30	0.17	0.210	0.260	0.077
18 h	2.30	2.19	1.81	2.24	0.21	0.565	0.202	0.249
30 h	3.69	3.48	2.97	3.14	0.22	0.041	0.390	0.340

[a,b] Difference in superscript indicates significance at P < 0.05.
[*] DMD-Dry matter degradation; NDFD-Neutral detergent fibre degradation; kd-Rate of degradation.
[**] AP 0-0% Dried apple pomace (DAP) inclusion; AP 25-25% DAP inclusion; AP 50-50% DAP inclusion; AP 75-75% DAP inclusion.

4.4 Discussion

The kikuyu pasture grazed during the trial had an average CP content of 196 g/kg DM, which is slightly below what had been observed by other researchers (Van der Colf, 2011; Cawood, 2016); however, CP content is variable depending on fertilisation practices and length of the grazing cycle. The ME content of 8.2 MJ/kg DM was within the range reported by Van Der Colf, 2011, but the NDF content of 450 g/kg DM was lower. There were no differences in pasture DMI between cows receiving the different concentrate supplements. The discrepancy between estimated pasture DMI using the RPM method compared to the TiO_2 method is due to the high inaccuracy of the RPM method, with almost 19% error. There was no increase in pasture substitution rate when a high NDF product such as DAP was fed as a supplement for cows grazing kikuyu even when the high NDF content of the AP 75 concentrate supplement resulted in a higher total NDF intake. As such the total daily DMI was similar between treatments, ranging between 12.9 and 14.2 kg/cow or 3.27 and 3.43% DMI as % BW. Depending on stage of lactation, average DMI ranges from 2.8 to 3.2% BW (McDonald *et al.*, 2010) and it can be assumed that all the cows in the trial consumed adequate feed according to BW. The substitution of pasture when a concentrate supplement is fed is normal and to be expected, with substitution rate increasing by 0.03-0.09 kg DM for every kg DM concentrate

supplement consumed (Stockdale, 2000; Kellaway & Harrington, 2004; McEvoy *et al.*, 2008). In previous studies, it has been shown that feeding concentrate supplements high in fibre did not lower pasture DMI, due to the low peNDF of the fibre component (Delahoy *et al.*, 2003; Sayers *et al.*, 2003).

Treatment did not have an effect on milk yield, as reflected in similar total ME intake and daily DMI for cows on the different treatments. There was, however, a decrease in the 4% FCM yield as the level of DAP inclusion increased, with a yield difference of 1.2 kg between the AP 0 and AP 75 treatment. In previous studies, milk yield remained unchanged when maize was partially replaced with a high NDF concentrate supplement, such as DAP, changing the starch to sugar ratio (O'Mara *et al.*, 1997; Leiva *et al.*, 2000; Cherney *et al.*, 2003; Delahoy *et al.*, 2003; Broderick *et al.*, 2008). There were also no differences observed in the fat content of milk; however, compounded with the milk yield that did not differ either, but showed a linear tendency, the fat yield and 4% FCM was lower for treatments AP 50 and AP 75. The decrease in 4% FCM and fat yield as the level of DAP inclusion increased is also reflected in the energy secreted via the milk, which was lower for cows on the AP 50 and AP 75 treatments compared to cows on the AP 0 treatments. An increase in milk fat was expected for cows receiving higher levels of DAP due to the high NDF content and low starch content, but this was not realised. Various authors have documented an increase in milk fat content when high fibre concentrates are supplemented, as well as when sugar content increases (Leiva *et al.*, 2000; Broderick *et al.*, 2008; Penner & Oba, 2009; Higgs *et al.*, 2003). Treatment did not have any effect on milk protein content. In a study by Delahoy *et al.* (2003), the substitution of maize with beet pulp resulted in a decrease in the milk protein content. Similarly, Leiva *et al.* (2000) and Broderick *et al.* (2002) found a decrease in milk protein content when dried citrus pulp was used to substitute for maize. In other studies no changes in milk and protein content have been observed (McCormick *et al.*, 2001; Cherney *et al.*, 2003; Abdollahzadeh *et al.*, 2010). The information available is highly variable and dependent on specific circumstances. Basic principles can be applied from previous research stated above; however, no clear answers are available from the literature on the nature of DAP for cows grazing pasture.

The mean daily pH was lowest for cows receiving the AP 75 concentrate supplement; however, there was no difference in the amount of time that the rumen spent below pH 6.2, 6.0 and 5.8 and in the diurnal pH over a 24 hour period. Previous *in vivo* studies found that increasing the sugar content, thereby decreasing the starch content, did not negatively affect pH (Sutoh *et al.*, 1996; Hall & Herejk, 2001; Ribeiro *et al.*, 2005). This premise has been

confirmed in various TMR based trials where sugar content was increased at the expense of maize and where no change in ruminal pH was observed (Leiva *et al.*, 2000; Broderick & Radloff, 2004; Broderick *et al.*, 2008; Oelker *et al.*, 2009). As such, a decrease in ruminal pH was not expected; however, there were no differences in diurnal ruminal pH fluctuations. Furthermore, the high NDF content of the AP 75 concentrate supplement, would be thought to increase rumen pH, but the low peNDF nature of the DAP would limit its efficiency in this regard. There were also no differences in the VFA profile and the digestibility of pasture, both DMD and NDFD, indicating that even though the mean pH was lower, there were no adverse effects on rumen activity and rumen health. In previous studies where the effect of higher sugar content in the diet on rumen parameters was observed, increased acetate production was found (Bach *et al.*, 1999; Ribeiro *et al.*, 2005; Poulsen *et al.*, 2012) and no change in propionate production was seen (McCormick *et al.*, 2001). The VFA profile of the ruminal fluid does not offer an explanation for the high 4% FCM yield obtained. In general, a higher acetate concentration would be expected to lead to increased milk fat content, but this was not observed. Furthermore, the increased NDF consumption as found with cows receiving the AP 75 concentrate supplement was not reflected in the VFA profile, where increased NDF intake would usually increase the acetate concentration (Ribeiro *et al.*, 2005; McDonald *et al.*, 2010). The effect of replacing maize with dried molasses, dried citrus pulp or sucrose in TMR or *in vitro* systems varied from improving DMD and NDFD at certain times of incubation (Broderick & Radloff, 2004; Holtshausen *et al.*, 2004; Broderick *et al.*, 2008) to not having any effect (Malan, 2009; Penner & Oba, 2009).

4.5 Conclusion

The replacement of ground maize with DAP in the concentrate supplement fed to cows grazing kikuyu pasture led to a decrease in 4% FCM yield and fat yield; however, milk fat and milk protein contents remained unchanged. There were no differences in pasture DMI and no substitution of pasture occurred. The mean ruminal pH was lowest for cows receiving the AP 75 concentrate supplement. However, due to the fact that all mean pH values varied between approximately 6.0 and 6.2, there were no adverse effects on rumen functionality and the VFA profile and pasture DMD and NDFD did not differ between treatments. The use of DAP as an energy source for dairy cows grazing kikuyu pasture can be considered, but the possible decrease in 4% FCM yield should not be overlooked. The cost of DAP is usually lower than that of maize in the southern Cape of South Africa; however, it is a limited feed source and the

distance for transport could increase the price drastically. The partial substitution of ground maize with DAP appears to be most advantageous when considering the comparable milk production and lower cost of feed.

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Chapter 5: The effect of dried apple pomace as a replacer for maize in the concentrate for Jersey cows grazing ryegrass pasture on production and rumen metabolism

Abstract

Ryegrass pasture is characterised as having a high crude protein content (214-298 g/kg DM) but a low fibre content (390-550 g NDF/kg DM). Additional supplementation of energy based concentrates is provided to ensure profitable milk production. The high starch content in these supplements could negatively impact the rumen environment; lowering rumen pH and inhibiting pasture degradability. Dried apple pomace (DAP) is a possible alternative energy source to maize for dairy cows grazing pasture; however, little information is available on the effectiveness of this high fibre by-product for milk production from pastures. The aim of this study was to determine the potential use of DAP as an energy source for Jersey cows grazing ryegrass pasture. Seventy six lactating Jersey cows were blocked according to milk yield, days in milk and lactation number. Cows within blocks were randomly allocated to one of four treatments. Treatments were: NDAP-0% dried apple pomace and 75% maize; LDAP-25% dried apple pomace and 50% maize; MDAP-50% dried apple pomace and 25% maize; HDAP-75% dried apple pomace and 0% maize. Cows received 6 kg as is of the allocated concentrate in the milking parlour daily. Cows of all four treatments strip grazed perennial ryegrass pasture over an area of 8.6 ha. Additionally, four ruminally cannulated cows were used to monitor treatment effect on rumen activity and health. Each cow was randomly allocated to one of four treatments and subjected to a four period crossover. The 4% fat corrected milk yield was lower for cows in treatment HDAP than for cows in treatments NDAP and LDAP, differences ranging between 1.7 and 2.3 kg/day. The milk protein content was lowest for cows in treatments NDAP and MDAP, showing a cubic trend (P = 0.005), with milk fat content increasing as the level of maize substituted by DAP increased. Treatment had no effect on rumen metabolism parameters. Replacing maize with DAP is a viable option for cows grazing ryegrass pasture; however, the decrease in 4% fat corrected milk yield and the potential economic impact should not be overlooked.

5.1 Introduction

In the southern Cape of South Africa, ryegrass pasture (annual and/or perennial) is the most common pasture available for grazing during the winter and spring months (Botha *et al.*, 2008). Ryegrass pasture is characterised as a pasture high in CP content (214-298 g/kg DM; Joubert, 2012) but low in NDF content (390-550 g/kg DM; van der Colf, 2011). To ensure high production outputs of cows, additional energy sources are also fed, usually in the form of a high energy concentrate supplement (Allen & Knowlton, 1995; NRC, 2001). These supplements often contain high levels of maize as the main energy source, which is also high in starch. A high starch content has a limiting effect on the rumen microbial population due to the production of lactic acid; which is associated with the degradation of starch in the rumen, leading to a decrease in ruminal pH (Calsamiglia *et al.*, 2010; Poulsen *et al.*, 2012; Jacobs, 2014). The decreased microbial activity due to low ruminal pH also negatively affects pasture degradation and lowers VFA production, which impacts negatively on milk production. Alternatively, it could be beneficial for production to supplement cows that graze on ryegrass pasture with an energy source low in starch but high in sugar and NDF content, such as dried apple pomace (DAP). Dried apple pomace is a by-product of the apple juicing industry and contains (on a DM basis) 483–612 g/kg NDF, 7.8–11.6 MJ/ kg ME and 77–80 g/kg CP (NRC, 2001; Edwards & Parker, 1995; Mirzaei-Aghsaghali *et al.*, 2011). The chemical composition of DAP depends on the processing methods applied as well as the specific apple variety and how it was managed post-harvest (Kennedy *et al.*, 1999). A few production studies have evaluated the use of apple pomace, either dried or ensiled, for use as an energy source for dairy cows; however, those that are available were implemented on TMR systems (Edwards & Parker, 1995; Abdollahzadeh *et al.*, 2010) and limited information is available on DAP fed as the main energy source for pasture based cows. The aim of this study was to determine the potential use of DAP as an energy source for Jersey cows grazing ryegrass pasture.

5.2 Materials and methods

5.2.1 Location and general management

The study was carried out at the Outeniqua Research Farm, situated in the Western Cape province of South Africa (22º 25' 16" E and 33º 58' 38" S) during late winter and early spring, over a period of 84 days. The mean minimum and maximum temperatures and total rainfall during the study period were 9.63°C, 20.3°C and 135.9 mm, respectively (ARC, 2016). An

area of 8.6 ha of perennial ryegrass (*Lolium perenne*, cv. Arrow) planted into a kikuyu (*Pennisetum clandestinum*) pasture base at a seeding rate of 18 kg/ha using an Aitcheson seeder, was used as roughage source. Ryegrass pasture was predominantly available to cows (68%) instead of kikuyu (2.5%), which is mainly dormant during the winter months. The rest of the pasture consisted of 12.5% legumes and 17% other grasses. The study area used for grazing was fertilised with 42 kg of N/ha post-grazing using limestone ammonium nitrate (280 g N/kg) and the soil in this area was characteristic of a Witfontein soil form (Swanepoel et al., 2013). Ethical clearance was granted by the Research Ethics Committee of Stellenbosch University, South Africa (SU-ACUD15-00094).

5.2.2 Treatment description and experimental design

The study consisted of four treatments. Treatments where defined according to the level of DAP included into the concentrate supplement, creating a gradient of starch and sugar content. The treatment concentrates were also formulated to be isonitrogenous (110g CP/kg DM) and have similar mineral contents. Treatments were as follows (Table 5-1):

- NDAP - 0% Dried apple pomace in concentrate,

- LDAP - 25% Dried apple pomace in concentrate,

- MDAP - 50% Dried apple pomace in concentrate,

- HDAP - 75% Dried apple pomace in concentrate.

Seventy-six lactating Jersey cows were used for a production study where treatment effect on milk yield, milk composition, body weight (BW), body condition score (BCS) and pasture DMI were determined. These cows were blocked according to milk yield (mean ± SE) over the previous 3 weeks (18.4 ± 0.01 kg), days in milk (97.2 ± 0.27 d) and lactation number (3.79 ± 0.04). Treatments were then randomly assigned to one of four cows in each block, thus resulting in a complete randomised block design. An additional four ruminally cannulated cows were used in a rumen metabolism study, which ran in conjunction with and simultaneous to the production study. This component of the study was included to determine treatment effect on VFA and NH_3-N concentration in the rumen, the diurnal pH of the rumen and pasture degradability. Each ruminally cannulated cow was randomly allocated to one treatment and subjected to a four period crossover design. Fourteen days of adaptation to treatment was

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allowed before the rumen sampling period (7 days), after which treatment allocation crossed over and the next adaptation period commenced. The ruminally cannulated cows of the rumen metabolism study grazed with the cows in the production study and were subjected to the same experimental procedure with respect to feeding and milking. All cows received 6 kg as is of the respective treatment concentrate daily, which was offered in two portions of 3 kg DM each during the morning and afternoon milking sessions. A rising plate meter (RPM), along with the linear regression equation: $Y = 91.06*H–200.59$, where Y = DM yield and H = RPM height, was used to estimate the DM yield of pasture (Steyn, 2012) and mean daily pasture DMI of all the cows was calculated as the difference in pasture yield before and after grazing. Strip grazing was applied to ensure an estimated daily pasture DMI of 10 kg DM/cow and an average of 28 days grazing cycle was applied. Water was available *ab libitum*.

Table 5-1 Ingredient composition (g/kg DM) of the four concentrate supplements used in the study.

Parameter	Treatment[*]			
	NDAP	LDAP	MDAP	HDAP
Ground maize	750	500	250	0
Dried apple pomace	0	250	500	750
Soya oil cake meal	50	50	50	50
Wheat bran	145	122	98	75
Molasses (liquid)	20	40	60	80
Feed lime	25	23	21	19
Salt	6	6	6	6
Urea	0	3	6	9
Premix[**]	1	1	1	1
Magnesium oxide	3	3	3	3
Mono-Calcium Phosphate	0	2	5	7

[*] NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP.

[**] Premix-4 mg/kg copper; 10 mg/kg manganese; 20 mg/kg zinc; 0.34 mg/kg iodine; 0.2 mg/kg cobalt; 0.06 mg/kg selenium; 6 x 106 IU vitamin A; 1 x 106 IU vitamin D3; 8 x 103 IU vitamin E (Supplier: Cape Feed and Grain, George East, 6539, South Africa).

5.2.3 Sample collection and analysis

Production study. Cows were weighed and BCS at the commencement and completion of the trial. Cows were milked twice a day at 05:30 and 13:30. Milk yield was automatically recorded at each milking with the Dairy Master milking system for a period of 33 days. Composite milk samples of the morning and afternoon milk were collected every week during this period and samples preserved with Bronopol. Samples were then analysed for fat, protein, lactose, MUN, SCC and pH (FOSS CombiFossTM FT+; FOSS, Foss Allè 1, DK–3400 Hillerød, Denmark). The net energy for lactation of cows of the four different treatment groups was calculated as: NEL (Mcal/kg) = 0.0929 x Fat% + 0.0547 x Protein% + 0.0395 x Lactose% (NRC, 2001). Treatment specific pasture DMI of intact cows was determined with the use of

titanium oxide (TiO_2) as an internal marker. Ten cows of each treatment were dosed with 3g of TiO_2 twice a day for ten days (6 g TiO_2/day) and one additional cow per treatment was included for background analysis. The cows used were from the production study and were randomly selected (remained within blocks). Grab faecal samples were collected twice a day on days 6 to ten of the dosing period (Glindemann et al., 2009). Faecal samples were then dried at 60˚C for 72 hours (de Souza et al., 2015). After drying samples were pooled per cow and then ground through a 1 mm sieve. Faecal samples of dosed and background cows, pasture samples and feed samples were then analysed for TiO_2 content by the method of Myers et al. (2004). Total faecal excretion was then determined as: Total faecal excretion (g/day) = Daily dose of TiO_2 (g/day)/ TiO_2 concentration in faeces (g/day) (de Souza et al., 2015). The indigestibility of the concentrate supplement and pasture was determined after incubating samples for 240 h, with continuous infusion of CO_2 (Goering & van Soest, 1970). The residue remaining in the Dacron bags after incubation was regarded as the iNDF. Finally pasture DMI was determined with the following equation: Pasture DM intake (kg/day) = [{Total faecal excretion (kg/day) x iNDF of faeces (kg/kg)} - iNDF intake from concentrate (kg/day)]/ iNDF of forage (kg/kg) (Cabral et al., 2014).

Rumen metabolism study. During the rumen sampling periods, rumen fluid samples were collected from the four ruminally cannulated cows in eight hour time intervals; 06:00, 14:00, and 22:00; filtered through a double layer of cheesecloth after which an aliquot sample was placed in an airtight container and immediately frozen for later analysis of NH3-N (Broderick & Kang, 1980) and VFA (Filípek & Dvořák, 2009). Indwelling TruTrack pH Data Loggers (Model pH-HR mark 4, Intech Instruments LTD, New Zealand, www.intech.co.nz) were used for the recording of rumen pH values over a 72 hour period. Loggers were carefully calibrated with two buffer solutions (pH 4 and 9) and tested with a pH 7 buffer. Once calibration was completed, loggers were placed in a standard solution of pH 7 for 12 hours. Loggers were then inserted into the rumen for a 72 hour logging period. Once logging was complete, loggers were removed from the rumen and placed in a standard solution of pH 7 for 6 hours. Any drift in pH recorded was used to correct the pH data. Pasture dry matter degradability (DMD) and pasture NDF degradability (NDFD) were determined through the use of *in sacco* Ankom Dacron bags (10 x 20 cm), with a mean pore size of 53 µm, containing 5 g dried and cut (5 mm) ryegrass pasture samples. The Ankom Dacron bags were incubated for 6, 18 and 30 hours (Van Amburgh et al., 2003; Cruywagen, 2006). After incubation, all bags were washed in a twin tub washing machine and dried at 60˚C for 72 hours. Residue in bags was analysed for DM

(AOAC, 2002; method 934.01) and aNDFom (Robertson and van Soest, 1981; using the Ankom fibre analysis system) content. Pasture DMD and NDFD was then determined with the rate calculator of van Amburgh et al. (2003).

Feed and pasture samples. Feed and pasture samples were collected weekly, dried, pooled over two weeks and analysed for DM, OM (AOAC, 2002; method 942.05), CP (AOAC, 2002; method 990.03; using the Leco N analyser, model FP 528), aNDFom, ADFom and ADL(sa) (Robertson and van Soest, 1981; using the Ankom Fibre Analyzer, ANKOM Technology, Fairport, NY, USA). Samples were also analysed for NDIN (samples first analysed according to aNDF procedure, residue then analysed for N on Leco N analyser, model FP 528), ADIN (samples first analysed according to ADF procedure, residue then analysed for N on Leco N analyser, model FP 528), EE (AOAC, 2002; method 920.39), GE (MC 1000 Modular Calorimeter, Energy Instrumentation, Sandton, South Africa, 2146), IVOMD (Buys et al., 1996), starch (AOAC, 2002; method 996.11), sugar (AOAC, 1998; Method 982.14), Ca (ALASA, 1998; Method 6.1.1), P (ALASA, 1998; Method 6.1.1), K (ALASA, 1998; Method 6.1.1) and Mg (ALASA, 1998; Method 6.1.1). Metabolisable energy (ME) was calculated as: ME (MJ/kg DM) = GE x IVOMD x 0.82 (Robinson et al., 2004).

Statistical analysis. Milk production, milk composition, BW, BCS, energy efficiency and pasture DMI data were subjected to a mixed model procedure with main effects as treatment and block using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Polynomial contrasts were included to test for linear, quadratic and cubic effects. Covariance was not included due to the blocking of cows, which is expected to minimise variation, based on cow factors, between treatments. Volatile fatty acid and ammonia data were analysed using a Mixed Model Procedure over time, with polynomial contrasts also included. The *in sacco* Dacron bag study data were subjected to a main effects ANOVA and polynomial contrasts. The ruminal pH data were subjected to a repeated measures ANOVA and polynomial contrasts. Tukey's test was used to compare the treatment means at a 5% significance level. The null hypothesis was: Ho: Ho: $\mu1 = \mu2 = \mu3 = \mu a$. The null hypothesis was rejected where $P < 0.05$. Least squares means were used to calculate a pooled standard error of treatment means. Shapiro-Wilk tests were used to test for normality (Shapiro & Wilk, 1965).

5.3 Results

5.3.1 Feed composition and pasture DM intake

The ME content of the four concentrate supplements were comparable, ranging from 10.4 to 10.9 MJ/kg DM (Table 5-2). The CP content varied between 106 g/kg DM for the NDAP treatment to 119 g/kg DM for the HDAP treatment. As the sugar content increased with increased DAP inclusion, the starch content decreased. The increase in NDF content is also notable for increased DAP inclusion, ranging from 106 g/kg DM to 307 g/kg DM in the NDAP and HDAP treatments, respectively. Pasture chemical composition over the trial period is shown in Figure 5-1. Mean daily pasture DMI of all cows, calculated as the difference in pasture yield before (2065 kg DM/ha) and after grazing (411 kg DM/ha), was estimated at 10.6 kg DM/cow (Table 5-3). The average pre-grazing pasture height was 32 on the RPM and the average post-grazing height was 11.6 on the RPM, indicative of a well grazed pasture (Irvine et al., 2010). Among treatments pasture DMI was 2.2 – 3.5 kg DM lower (P = 0.003) for cows in the HDAP treatment, as determined by the TiO_2 method. Similarly the total DMI and DMI as % BW were also lower for these cows; P = 0.007 and P = 0.035, respectively (Table 5-4).

Table 5-2 Mean chemical composition (g/kg DM) of the four concentrate supplements, pasture and DAP used in the study (n = 3).

Parameter[*]	Treatment[**]				Pasture[***]	AP (n = 1)
	NDAP	LDAP	MDAP	HDAP		
DM	903	905	918	924	857	921
OM	949	946	942	936	898	981
CP	106	110	113	119	177	67.4
EE	13.1	17.7	24.3	25.4	32.9	37.1
NFC	724	650	571	484	344	435
Total sugar	35.2	72.3	101	152	73.4	115
Fructose	8.33	26.0	40.3	70.6	24.2	54.3
Glucose	5.55	18.5	31.0	46.1	11.6	52.5
Sucrose	21.3	26.9	31.0	36.7	37.6	8.20
Starch	749	576	296	135	69.9	91.0
NDF	106	169	233	307	344	442
ADF	36.1	103	182	259	231	354
ADL	5.24	21.0	43.2	59.9	18.3	134
IVOMD	876	850	829	782	829	785
GE (MJ/kg DM)	14.7	15.0	15.7	15.8	13.8	20.1
ME (MJ/kg DM)	10.8	10.7	10.9	10.4	95.7	13.2
Ca	11.0	10.9	11.9	16.2	3.60	1.43
P	4.21	3.56	4.13	4.37	4.32	1.54
Ca: P	2.62:1	3.07:1	2.89:1	3.71:1	0.85:1	0.93:1
Mg	3.31	3.08	3.21	3.29	2.71	0.99
K	6.74	7.57	8.36	9.64	32.4	6.50

[*] DM-Dry matter; OM-Organic matter; ME-Metabolisable energy; CP-Crude protein; EE-Ether extract; NFC-Non-fibrous carbohydrates; NDF-Neutral detergent fibre; ADF-Acid detergent fibre; ADL-Acid detergent fibre; IVOMD-In vitro organic matter digestibility; GE-Gross energy.

[**] NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP.

[***] Pasture–Annual Italian ryegrass (*Lolium multiforum*, variety italicum, cultivar Jeanne).
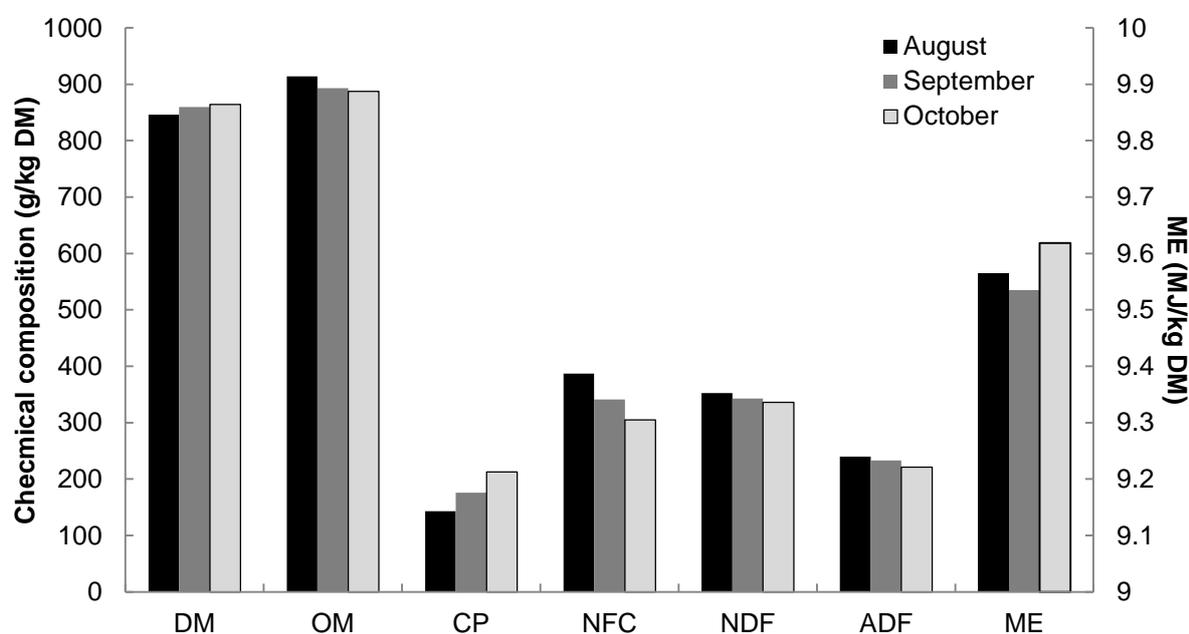
Figure 5-1 Chemical composition of pasture (Lolium multiforum) grazed from August to October 2016; DM – Dry matter; OM – Organic matter; CP – Crude protein; NFC – Non-fibrous carbohydrates; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; ME - Metabolisable energy.

Table 5-3 The pre- and post-grazing RPM height (mean ± SD), pasture yield, pasture allowance and pasture DM intake determined using the seasonal linear regression.

Parameter[*]	Pasture values
Pre-grazing	
RPM height	32 ± 4.81
Pasture yield (kg DM/ha)[**]	2065 ± 390
Daily pasture allowance (kg DM/cow)	9.6 ± 1.56
Post-grazing	
RPM height	11.6 ± 1.24
Pasture yield (kg DM/ha)	411 ± 90.9
Estimated daily pasture DM intake (kg DM/cow)	10.6 ± 2.28

[*] RPM-Rising plate meter; DM-Dry matter.
[**] $Y = 81.1*H\text{-}544$; where Y = DM yield and H = RPM height reading (R^2=0.81).

Table 5-4 Pasture DMI in relation to cow BW determined using TiO_2 as an internal marker (n = 10)

Parameter[*]	Treatment[**]				SEM	P-value		
	NDAP	LDAP	MDAP	HDAP		Linear	Quadratic	Cubic
Faecal excretion (kg/d)	3.38	3.38	3.74	3.61	0.14	0.103	0.634	0.173
Pasture DMI (kg/d)	12.5[a]	11.2[a]	11.7[a]	9.01[b]	0.73	0.005	0339	0.154
Total DMI (kg/d)	17.9[a]	16.7[a]	17.2[a]	14.5[b]	0.73	0.007	0.345	0.145
BW (kg)	398	381	393	383	12.4	0.542	0.769	0.352
DMI as % BW (kg DM)	4.50[a]	4.38[ab]	4.42[ab]	3.83[b]	0.20	0.035	0.263	0.388
NDF intake as % BW (kg DM)	1.22	1.25	1.36	1.26	0.07	0.441	0.345	0.368

[a,b] Difference in superscript indicates significance at $P < 0.05$.
[*] DMI-Dry matter intake; BW-Body weight; NDF-Neutral detergent fibre.
[**] NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP.

5.3.2 Milk production and composition

Milk yield showed a linear trend (P = 0.028), with milk yield decreasing as the level of DAP increased. The 4% FCM yield was lower for cows in treatment HDAP than for cows in treatments NDAP and LDAP and for cows in treatment MDAP compared to cows in treatment NDAP (P <0.001; Table 5-5), with differences ranging between 1.7 and 2.3 kg/day. Fat yield was lower for cows in treatments MDAP and HDAP compared to cows in treatments NDAP and LDAP, corresponding to the decrease in milk fat content, P <0.001 and P = 0.026, respectively. Milk protein yield decreased linearly as the level DAP inclusion increased, whereas milk protein content was lowest for cows in treatments NDAP and MDAP, showing a cubic trend (P = 0.005). Cows in the NDACP treatment had lower lactose content than cows in treatments LDAP, MDAP and HDAP (P = 0.004). The MUN was lowest for cows in the NDAP treatment. Treatment had no effect on BW change, ADG or BCS change. The total daily

ME intake was lowest for cows in the HDAP treatment (P = 0.005). The energy excreted via one kg of milk was highest for cows in treatment LDAP (P = 0.008), while the total daily energy excreted via milk was lowest for cows in treatment HDAP, compared to cows on treatments NDAP and LDAP (P = 0.005; Table 5-6). Overall, treatment did not have an effect on the conversion efficiency of feed energy to milk energy.

Table 5-5 Mean milk yield, milk composition and BW and BCS change of cows receiving one of four concentrate supplements (n = 19).

Parameter[*]	Treatment[**]				SEM	P-value		
	NDAP	LDAP	MDAP	HDAP		Linear	Quadratic	Cubic
Production (kg/cow)								
Milk yield	19.1	18.3	18.6	17.4	0.63	0.028	0.762	0.227
4% FCM yield	20.9[a]	20.5[ab]	19.2[bc]	18.6[c]	0.64	<0.001	0.783	0.485
Fat yield	0.88[a]	0.88[a]	0.79[b]	0.78[b]	0.03	<0.001	0.818	0.089
Protein yield	0.68	0.68	0.66	0.63	0.02	0.012	0.317	0.793
Milk composition								
Fat (g/kg)	46.2[ab]	48.2[a]	42.8[c]	44.8[bc]	1.00	0.026	0.998	0.001
Protein (g/kg)	35.8[a]	37.6[b]	35.8[a]	36.4[ab]	0.53	0.983	0.189	0.005
Lactose (g/kg)	46.4[a]	47.5[b]	47.4[b]	47.6[b]	0.26	0.004	0.056	0.229
SCC (x 10^3 cells/mL)	140	142	217	196	50.9	0.264	0.821	0.432
MUN (mg/dL)	11.3[a]	12.6[b]	12.6[b]	12.6[b]	0.36	0.022	0.061	0.369
BW (kg)								
Before	404	404	411	403	9.06	0.945	0.641	0.560
Change	-6.67	-9.00	-10.4	-9.53	3.27	0.453	0.586	0.939
ADG	-0.14	-0.20	-0.24	-0.21	0.07	0.454	0.567	0.942
BCS (Scale 1-5)								
Before	2.03	2.05	2.05	2.09	0.02	0.066	0.781	0.534
Change	0.16	0.13	0.22	0.17	0.04	0.441	0.730	0.127

[a,b] Difference in superscript indicates significance at P <0.05.

[*] FCM-Fat corrected milk; SCC-Somatic cell count; MUN-Milk urea nitrogen; BW-Body weight; ADG-Average daily gain; BCS-Body condition score.

[**] NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP.

Table 5-6 Mean daily energy intake and excretion (milk) and conversion of energy into milk of cows receiving one of four concentrate supplements (n = 10).

Parameter[*]	Treatment[**]				SEM	P-value		
	NDAP	LDAP	MDAP	HDAP		Linear	Quadratic	Cubic
Total ME intake (MJ/cow)	178[a]	166[a]	172[a]	144[b]	6.94	0.005	0.263	0.106
NE_L (MJ/L) in milk	3.29[a]	3.48[b]	3.22[a]	3.24[a]	0.06	0.125	0.185	0.008
Energy in milk (MJ/day)	67.0[a]	67.9[a]	63.2[ab]	57.8[b]	3.12	0.005	0.190	0.638
Conversion of ME intake to milk energy (%)	37.6	41.4	37.3	41.1	2.22	0.467	0.984	0.080
Kg milk/kg feed	1.14	1.19	1.17	1.25	0.07	0.260	0.798	0.576

[a,b] Difference in superscript indicates significance at P <0.05.

[*] NE_L-Net energy for lactation (Mcal/kg) = 0.0929 x Fat% + 0.0547 x Protein% + 0.0395 x Lactose% (NRC, 2001); 1 Mcal = 4.184 MJ.

[**] NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP.

5.3.3 Rumen metabolism parameters

No differences in diurnal ruminal pH were recorded (Figure 5-2) and the mean pH over a 24 h period was also not affected (Table 5-7). The time that ruminal pH was below pH 6.2, 6.0 and 5.8 did not differ among treatments; however, there was a quadratic trend for a longer time below pH 5.8 for cows in treatments NDAP and HDAP (P = 0.023). Treatment had no effect on individual VFA concentrations or the acetate to propionate ratio. There was a cubic trend for acetate concentration, where cows in treatments LDAP and HDAP had a higher acetate concentration than cows in NDAP and MDAP treatments (P = 0.035). The NH_3-N concentration did not differ between treatments. Pasture DMD and NDFD were also determined at 6, 18 and 30 hours of incubation (Table 5-8). No differences in degradability or rate of degradation were observed.
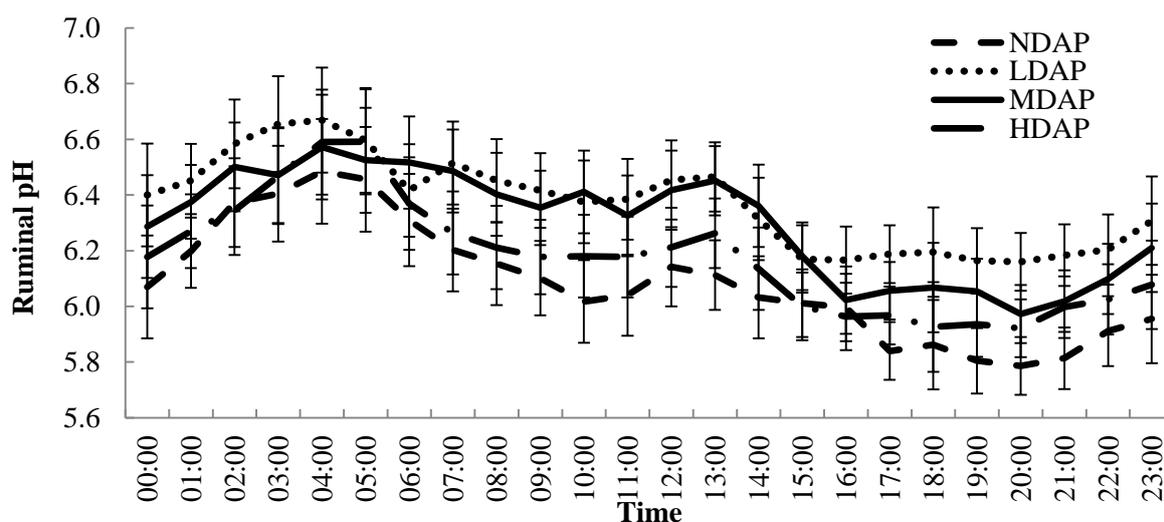

Figure 5-2 Diurnal fluctuations in ruminal pH of cows (n = 8) receiving one of four concentrate supplements; error bars represent SEM, NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP.

Table 5-7 Mean ruminal pH and time that the rumen spent below pH 6.2, 6.0 and 5.8, individual VFA concentrations and mean NH₃-N concentration of cows receiving one of four concentrate supplements.

Parameter[*]	Treatment[**]				SEM	P-value		
	NDAP	LDAP	MDAP	HDAP		Linear	Quadratic	Cubic
Mean pH	6.09	6.37	6.30	6.18	0.13	0.730	0.157	0.641
Time below (hrs)								
pH 5.8	6.63	4.38	4.38	8.5	1.05	0.277	0.023	0.704
pH 6.0	9.63	9.00	6.00	10.5	1.17	0.945	0.071	0.108
pH 6.2	13.3	10.6	10.1	11.3	1.68	0.419	0.306	0.949
VFA (mM/L)								
Acetate	52.8	63.0	55.7	58.1	2.24	0.430	0.132	0.035
Propionate	16.7	19.1	17.0	17.1	0.98	0.848	0.300	0.161
Butyrate	12.0	13.5	12.5	12.2	0.79	0.901	0.324	0.380
Iso-butyrate	1.28	1.43	1.26	1.56	0.14	0.406	0.411	0.546
Iso-valerate	0.74	0.80	0.70	0.62	0.08	0.283	0.460	0.653
Valerate	0.46	0.50	0.44	0.43	0.03	0.337	0.410	0.369
Acetate:Propionate	3.28	3.34	3.30	3.34	0.18	0.865	0.941	0.813
NH₃-N (mM/L)	9.13	9.49	10.8	10.0	0.74	0.231	0.327	0.257

[a,b] Difference in superscript indicates significance at P < 0.05.

[*] VFA-Volatile fatty acids; NH3-N-Ammonia nitrogen

[**] NDAP-0% dried apple pomace; LDAP-25% dried apple pomace; MDAP-50% dried apple pomace; HDAP-75% dried apple pomace.

Table 5-8 *In vivo* ryegrass pasture degradability parameters (n = 4) calculated with the rate calculator of van Amburgh *et al.* (2003) using *in sacco* Dacron bags at 6, 18 and 30 hours of incubation.

Parameter[*]	Treatment[**]				SEM	P-value		
	NDAP	LDAP	MDAP	HDAP		Linear	Quadratic	Cubic
DMD (%)								
6 h	45.4	45.5	46.2	42.7	1.40	0.293	0.255	0.452
18 h	71.6	73.0	72.4	71.3	1.75	0.848	0.523	0.861
30 h	84.7	84.0	84.3	84.7	1.07	0.945	0.590	0.878
NDFD (%)								
6 h	41.2	41.7	42.0	38.4	1.66	0.325	0.266	0.654
18 h	68.0	68.8	68.3	68.0	1.91	0.939	0.769	0.870
30 h	84.7	81.5	82.0	82.3	1.05	0.200	0.152	0.471
kd (%/h)								
6 h	9.85	10.0	10.1	8.95	0.57	0.346	0.275	0.644
18 h	6.19	6.44	6.24	6.71	0.65	0.652	0.880	0.937
30 h	8.61	6.54	7.25	7.36	0.70	0.370	0.169	0.323

[a,b] Difference in superscript indicates significance at P < 0.05.

[*] DMD-Dry matter degradation; NDFD-Neutral detergent fibre degradation; kd-Rate of degradation.

[**] NDAP-0% dried apple pomace; LDAP-25% dried apple pomace; MDAP-50% dried apple pomace; HDAP-75% dried apple pomace.

5.4 Discussion

The high fibre nature of the DAP is clear when the four treatment concentrates are compared, with the NDF content increasing incrementally from 106 g/kg DM in the NDAP treatment to 307 g/kg DM in the HDAP treatment. Cows in the HDAP treatment consumed only 9.01 kg of pasture, whereas cows in the NDAP, LDAP and MDAP treatments consumed between 11.2 and 12.5 kg pasture. Overall, cows in the HDAP treatment also consumed less DM per kg BW than cows in all other treatments, showing that pasture substitution took place to some extent. The extent of pasture substitution depends on the concentrate supplement being fed as well as the degradability of pasture, which would affect rumen fill. Lowered rumen pH may have a negative effect on NDF degradation, thus slowing the passage rate of feed, increasing rumen fill and may finally result in lowered pasture DMI (Bargo et al., 2003; Kellaway & Harrington, 2004). Treatment; however, had no effect on the mean rumen pH or the diurnal fluctuation in pH; pasture DMD and NDFD also remained similar between treatments, thus not providing an explanation for the decreased pasture DMI seen with cows receiving the HDAP treatment concentrate. Furthermore, Meijs (1986), Delahoy et al. (2003), Sayers et al. (2003) and Higgs et al. (2013) found that pasture substitution did not occur when high fibre based concentrate supplements, such as sugar beet pulp, citrus pulp and soybean hulls, were fed compared to high starch based concentrate supplements. As such, the high fibre nature of DAP was not expected to lower pasture DMI compared to other feed sources. Total NDF intake per kg BW was also not affected by treatment, showing that despite pasture DMI differences, NDF intake remained unchanged relative to BW. Total NDF intake per kg BW was also similar to values obtained by Kolver & Muller (1998), where NDF intake ranged between 1.2 and 1.5% of BW.

Total daily ME intake was lowest for cows in the HDAP treatment, even though the ME content of the four treatment concentrates were very similar (10.4-10.9 MJ/kg DM) and was due to the lower pasture DMI of cows in this treatment. The low pasture DMI and low total daily ME intake resulted in a trend for lower milk yield as DAP was substituted for maize and lowered 4% FCM yield for cows in the HDAP treatment compared to cows in the NDAP and LDAP treatments, indicative of an energy shortage. According to Bargo et al. (2003) pasture substitution rate and milk response are negatively correlated, with milk yield decreasing as the substitution rate increases, as was seen here. In a previous study, the use of DAP as a substitute to maize did not result in lowered pasture DMI, but 4% FCM was also lower (Steyn et al., 2017

unpublished data). Similarly, cows in the HDAP treatment also secreted less energy in milk, corresponding to the lower 4% FCM and fat yield. The composition of milk was influenced by treatment in various ways. Fat content was lowest for cows in the MDAP treatment and highest for cows in the LDAP treatment. Milk composition often decreases on a winter pasture type such as perennial ryegrass, due to the inherent low NDF content (McDonald et al., 2010; Sairanen et al., 2006); however, the high fibre nature of DAP was expected to contribute to improved milk fat content. As such, a linear trend was expected with milk fat content increasing as the level of DAP substituted for maize increased. No increase in acetate production was found, corresponding to the similar NDF intake per kg BW. In a study by Steyn et al. (2014), the feeding of a high fibre concentrate supplement to cows on ryegrass pasture yielded a ruminal acetate concentration of 75.1 mM/L, corresponding to a milk fat content of 49.2 g/kg. In this study, ruminal acetate concentration ranged from 52.8 to 63.0 mM/L, with no differences between treatments and no difference in acetate: propionate ratio. In a continuous culture system study by Ribeiro et al. (2005), the addition of sugar to the diet increased acetate production. Similarly, the use of beet pulp over cracked maize in a pasture-based *in vitro* increased the production of acetate (Bach et al., 1999). In a study by McCormick et al. (2001) no difference in acetate production was found when sucrose was supplemented *in vitro*. The effect of the sugar to starch ratio of feed on acetate concentration is confounding and does not provide a clear guideline of what is to be expected. Even though the NDF intake per kg BW was similar between treatments, the increasing NDF content as the level DAP inclusion increases would be expected to increase as well due to the high correlation of acetate concentration to milk fat content (Kennelly & Glimm, 1998; Seymour et al., 2005); however, this was not found. These VFA results fail to explain the lowered milk fat content in cows receiving the MDAP and HDAP treatment concentrates. Protein yield decreased linearly as the level of DAP substitution for maize increased and milk protein content showed a cubic response. Generally, milk protein levels do not respond readily to dietary manipulation (Bargo et al., 2003; Kellaway & Harrington, 2004) and as such no major treatment effects were expected. The milk protein content observed during this study falls within the average range of milk protein content for Jersey cows, 3.4-3.9% (Bargo et al., 2003; Erasmus, 2009; Steyn et al., 2014). There was no difference in BW before or BW change between treatments; however, all cows had a negative ADG for the duration of the study, indicating that they were still in a negative energy balance as seen in early- to mid-lactation (NRC, 2001). Cows in the HDAP treatment did not compensate for lower ME intake by mobilizing body reserves to a greater

extent than cows in the NDAP, LDAP and MDAP treatments, rather milk yield suffered.

The rumen environment of cows in the HDAP treatment did not show any variation in diurnal rumen pH fluctuations or the mean ruminal pH in response to a higher concentrate: pasture ratio. This could be due to the high NDF content of the HDAP concentrate treatment as well as the lower digestibility, avoiding over supply of rapidly digested energy to the microbes (Oba, 2011). Furthermore, the low starch content of the HDAP treatment concentrate (135 g/kg DM) would contribute to a more stable ruminal pH. In comparison to starch, sugar provides less C for VFA production per unit of mass (Hall & Herejk, 2001) and micro-organisms are able to convert sugar to glycogen for short term storage (Hall & Weimer, 2007), essentially temporarily reducing VFA production in the rumen (Oba, 2011) and reducing the potential negative effect of high sugar on the rumen pH. In previous studies it has been found that high sugar levels at the expense of starch did not negatively affect ruminal pH (Broderick & Radloff, 2004; Broderick et al., 2008; Oelker et al., 2009). The MUN content of milk was lowest for cows receiving the NADP treatment concentrate; however, it was still in the acceptable range of 8-12 mg/dL (Kohn, 2007). Furthermore, there were no irregularities in the NH3-N levels in the rumen and levels were sufficient to maintain healthy rumen activity as well as supply N for milk protein production (Khalili & Sairanen, 2000; Kolver, 2003).

5.5 Conclusion

The incremental replacement of maize with DAP in the concentrate fed to Jersey cows grazing ryegrass pasture resulted in a decrease in 4% FCM yield, possibly due to lower pasture DMI and lower daily ME intake. Treatment did not have any effect on rumen metabolism, neither increasing nor decreasing pasture degradability. The lack of production response should be considered before recommending the degree to which maize is replaced with DAP in a concentrate supplement as this could have severe financial implications. Alternatively, DAP can often be procured at lower costs than maize, making it a potentially viable feed source under certain circumstances. The quality and availability of DAP is not very stable or reliable; however, in a country such as South Africa where 920 000 tons of apples are produced annually, of which 30% are purchased for further processing, there is a lot of room for improving the processing procedures and the supply of DAP. The potential antioxidant effects of phenolic compounds present in apple pomace warrants further investigation into the use and application of DAP on farm. Dried apple pomace should not be overlooked as a feed source for Jersey cattle grazing ryegrass pasture.

5.6 References

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Chapter 6: Ruminal bacterial community dynamics of cows grazing kikuyu-ryegrass pasture supplemented with maize and dried apple pomace

Abstract

The popularity of pasture-based dairy systems is increasing in South Africa and is the most common system used in the southern Cape of South Africa. Little to no ruminal bacterial community dynamics work has been done for dairy cows grazing pasture. Two in depth studies have been carried out to investigate the possibility of replacing ground maize with dried apple pomace in the concentrate supplement fed to cows on summer and winter pastures. This provided an opportunity to investigate the ruminal bacterial community dynamics of Jersey cows grazing two different pasture types, over two seasons and receiving different levels of fibre, sugar and starch in supplemental feeding. The aim of the study was to investigate whether ruminal bacterial community dynamics were affected by different inclusion levels of ground maize and dried apple pomace fed in a concentrate supplement to cows grazing kikuyu-ryegrass pasture. In both trials cows received concentrate supplements where ground maize was replaced incrementally to yield the following treatments: Trial A - AP 0: 0% dried apple pomace inclusion, AP 25: 25% dried apple pomace inclusion, AP 50: 50% dried apple pomace inclusion and AP 75: 75% dried apple pomace inclusion. Trial B - NDAP: 0% dried apple pomace inclusion, LDAP: 25% dried apple pomace inclusion, MDAP: 50% dried apple pomace inclusion and HDAP: 75% dried apple pomace inclusion. The experimental diets were similar over the two trials, except for slight differences in formulation in response to seasonal requirements and limitations of pasture. Cows in trial A grazed kikuyu pasture and cows in trial B grazed ryegrass pasture. There were significant bacterial community shifts between feeding high starch and low fibre concentrates compared to high sugar and high fibre concentrates, on both the summer and winter pastures. There were 16 phyla identified across both trials, with *Bacteroidetes*, *Firmicutes*, *Tenericutes* and *Proteobacteria* making up 92-98% of the total population. Higher variation was found within families, with 139 families observed in trial A and 145 families observed in trial B. It was concluded that the inclusion level of DAP did affect the ruminal bacterial community dynamics across pasture type.

6.1 Introduction

The popularity of pasture-based dairy systems is increasing in South Africa, with production from pasture-based areas also increasing (Lactodata, 2017). This is especially true for the southern Cape of South Africa, which produces the most milk from pastures, highlighting the importance of a deeper understanding of what drives the production of dairy cows in these systems. Little to no ruminal bacterial community dynamics work has been done for dairy cows grazing pasture, not even specifying breed, area or pasture type. Most sequencing work has investigated the ruminal bacterial community dynamics of beef steers, heifers and cows (Guan *et al.*, 2008; Callaway *et al.*, 2010; Fernando *et al.*, 2010; Pitta *et al.*, 2010; Broadway *et al.*, 2012), especially pertaining to adaptation to feedlot diets and a few have investigated the ruminal bacterial community dynamics of dairy cows, but mostly in TMR systems (Weimer *et al.*, 1999; Tajima *et al.*, 2001; Li *et al.*, 2009; Palmonari *et al.*, 2010; Welkie *et al.*, 2010; Jewell *et al.*, 2015). Within a pasture-based system there is also a lot of variation pertaining to cow breed, area, type of pasture and supplemental feeding. Kikuyu (*Pennisetum clandestinum*) pasture is very persistent in the southern Cape area and methods of maximising production off this pasture throughout the year include over-sowing with ryegrass pasture (*Lolium perenne* or *Lolium multiform*) (Botha *et al.*, 2008; van der Colf, 2011). There has also been a trend towards supplementing high fibre based concentrates, which are often more affordable due to lower maize inclusion and are less harmful to the rumen microbiome due to lower starch inclusion, avoiding sharp decreases in ruminal pH (Steyn *et al.*, 2014; Cawood, 2016). Dried apple pomace (DAP) is a by-product of the apple juicing industry and is high in fibre and sugar and low in starch. Two in-depth studies have been done to investigate the possibility of replacing ground maize with DAP in the concentrate supplement fed to cows on summer and winter pastures (Steyn *et al.*, 2017a and b). This provided an opportunity to investigate the ruminal bacterial community dynamics of Jersey cows grazing two different pasture types, over two seasons and receiving different levels of fibre, sugar and starch in supplemental feeding. As such, the aim of the study was to determine the effect of different inclusion levels of ground maize and DAP on the ruminal bacterial community dynamics for Jersey cows fed a concentrate supplement on kikuyu-ryegrass pasture.

6.2 Materials and methods

6.2.1 Experimental design

Two trials were carried out on the Outeniqua Research Farm, George, South Africa (22º 25' 16"E and 33º 58' 38"S). Trial A was carried out during March to May 2016 on kikuyu pasture (*Pennisetum clandestinum*) (Steyn et al, 2017a unpublished data). Trial B was carried out during August to November 2016 on perennial ryegrass (*Lolium perenne*, cv. Arrow) (Steyn et al, 2017b unpublished data). Treatments were defined according to the level of DAP included into the concentrate. Treatments were similar in both trials with regards to the ground maize and DAP inclusion; however, formulation differed slightly due to seasonal effects of pasture (Table 6-1). In trial A treatments were: AP 0: 0% DAP inclusion, AP 25: 25% DAP inclusion, AP 50: 50% DAP inclusion and AP 75: 75% DAP inclusion. In trial B treatments were: NDAP: 0% DAP inclusion, LDAP: 25% DAP inclusion, MDAP: 50% DAP inclusion and HDAP: 75% DAP inclusion. All treatments were formulated to be iso-nitrogenous and have a similar ME content, within trial A and B. Four ruminally cannulated cows were used in each of the trials. Each cow was randomly allocated to one of the four treatments in both trials. A 14 day adaptation period was allowed before crossing over to a new treatment. Once the sampling procedures were complete treatments were reallocated, following a 4 period crossover design. Cows received 6 kg as is/day of the concentrate supplement and were milked twice a day. After milking cows were allocated fresh pasture as strip grazing and water was available at all times. The rising plate meter (RPM) was used to allocate pasture to cows. Each trial made use of different cows and no cows were used in both trials.

6.2.2 Sample collection

Rumen fluid for DNA sequencing was sampled using a handheld suction pump inserted through a small hole in the rumen cannula. Samples were collected during three time intervals during the day (06:00, 14:00 and 22:00). At each collection session about 100 mL rumen fluid was collected per cow. This was then filtered through two layers of cheese cloth and a 30 mL aliquot sample was stored in a plastic container, which was immediately frozen at -20˚C. Samples were pooled per day, yielding a representative rumen fluid sample over a 24 hour period.

Additional rumen fluid was also sampled at the same times for analyses of VFA and NH_3-N concentration. Indwelling pH loggers were also used to determine the diurnal fluctuations of

ruminal pH. The full details pertaining to VFA, NH$_3$-N and pH data are available in Steyn *et al.*, 2017 a and b.

Table 6-1 Ingredient and chemical composition of the different concentrate supplements and pastures fed in trial A and B.

Parameter[*]	Trial A[**]				Trial B[***]					
	AP 0	AP 25	AP 50	AP 75	Kikuyu	NDAP	LDAP	MDAP	HDAP	Ryegrass
Ingredient (g/kg)										
Ground maize	750	500	250	0		750	500	250	0	
DAP	0	250	500	750		0	250	500	750	
Soybean oilcake	125	125	125	125		50	50	50	50	
Wheat bran	70	47	24	0		145	122	98	75	
Molasses (liquid)	20	40	60	81		20	40	60	80	
Feed lime	21	19	17	16		25	23	21	19	
Salt	6.0	6.0	6.0	6.0		6.0	6.0	6.0	6.0	
Urea	3.0	6.0	8.0	11		0	3.0	6.0	9.0	
Premix[****]	1.0	1.0	1.0	1.0		1.0	1.0	1.0	1.0	
MgO	3.0	3.0	3.0	3.0		3.0	3.0	3.0	3.0	
Mono-CaP	1.0	3.0	6.0	7.0		0	2.0	5.0	7.0	
Chemical (g/kg DM)										
CP	135	142	141	141	196	106	110	113	119	177
NFC	627	586	526	452	216	724	650	571	484	344
Total sugar	31.0	68.8	115	148	179	35.2	72.3	101	152	73.4
Fructose	4.60	21.1	46.0	72.9	17.2	8.33	26.0	40.3	70.6	24.2
Glucose	3.60	14.7	28.5	37.8	7.2	5.55	18.5	31.0	46.1	11.6
Sucrose	22.8	33.0	40.5	37.8	14.5	21.3	26.9	31.0	36.7	37.6
Starch	656	550	328	107	41.2	749	576	296	135	69.9
NDF	164	169	238	317	450	106	169	233	307	344
IVOMD	885	862	833	784	652	876	850	829	782	829
ME (MJ/kg DM)	11.8	11.7	11.8	11.4	8.17	10.8	10.7	10.9	10.4	95.7

[*] DAP-Dried apple pomace; DM-Dry matter; CP-Crude protein; NDF-Neutral detergent fibre; IVOMD-In vitro organic matter degradability; ME-Metabolisable energy.
[**] AP 0: 0% dried apple pomace (DAP) inclusion; AP 25: 25% DAP inclusion; AP 50: 50% DAP inclusion; AP 75: 75% DAP inclusion; *Pennisetum clandestinum*..
[***] NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP: *Lolium perenne*.
[****] Premix-4 mg/kg Cu; 10 mg/kg Mn; 20 mg/kg Zn; 0.34 mg/kg I; 0.2 mg/kg Co; 0.06 mg/kg Se; 6 x 106 IU vitamin A; 1 x 106 IU vitamin D3; 8 x 103 IU vitamin E.

6.2.3 DNA extraction and purification

Samples were thawed and centrifuged for 40 min at 8000 rpm. The supernatant was decanted and the cell pellet was re-suspended in extraction buffer. The procedure followed for bead beating, DNA clean up and DNA precipitation was that of Stevenson & Weimer (2007). After initial resuspension of the cell pellet in the extraction buffer, 1 mL was removed and added to 0.5 g of 100 µm Zirconium beads (OPS Diagnostics LLC, 291 Route 22 East, Building 6, Lebanon, NJ 08833, USA). A 20% SDS solution and phenol was then also added, at which point samples were bead beat for 2 minutes (Tissue Lyser LT, Qiagen, Hilden, Germany). After bead beating, samples were placed in a water bath for 10 minutes at 60°C, after which they were bead beat again for a further 2 minutes. The addition of heat to the bead beating process

increases the degrading ability of phenol, ensuring maximum breakdown of bacterial cell walls and exposure of DNA material. Following the bead beating process a series of DNA extractions/ purification steps were performed; removing any cell wall materials, lipids and plant debris. After DNA extraction was complete, DNA was precipitated and centrifuged for 20 minutes, resulting in a DNA pellet. These DNA pellets were then stored frozen until further processing.

6.2.4 DNA quality control

Before PCR and sequencing the extracted DNA samples were tested for total dsDNA content by means of the Qubit dsDNA HS assay (ThermoFisher Scientific, Catalogue number: 32854, Lot number: 1735144). Samples were also tested for the presence and amount of bacterial DNA with the Femto^{TM} Bacterial DNA Quantification Kit (Zymo Research, Catalogue number: E2006, Lot number: ZRC189387).

6.2.5 PCR and DNA sequencing

The DNA sequencing was done at the Central Analytical Facility, Stellenbosch University. The amplification of the 16S hypervariable regions, purification of the PCR amplicons and subsequent library building were all done according to the method described in the Ion 16S^{TM} Metagenomics Kit User Guide (2015). The 16S^{TM} Ion Metagenomics kit and Ion reporter were used for the sequencing and analyses of results. Briefly, two primer pools were used to amplify seven hypervariable regions (V2, V3, V4, V6, V7, V8 and V9) of bacterial 16S rRNA as supplied by the 16S^{TM} Metagenomics kit. Single flow reads were done with 850 cycles, managing to read lengths of up to 400 base pairs. The validity of reads was determined by read abundance and reads were only accepted if there were 10 or more unique reads. The identification of genus was cut off for reads below a 97% match and species below a 99% match. Primers were detected at each end and thus there was no read length filters applied. The library build was done with the use of two data sets, namely Curated MicroSEQ® 16S Reference Library v2013.1 and Curated Greengenes V13.5.

6.2.6 Statistical analyses

The ruminal pH data were subjected to repeated measures ANOVA and polynomial contrasts were included to test for linear, quadratic and cubic trends with SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Population dynamics data were subjected to Mixed Model

procedure with Cow and Treatments as main effects. Polynomial contrasts were also included here to identify any trends. Tukey's test was used to compare the treatment means at a 5% significance level. The null hypothesis was: Ho: $\mu_1 = \mu_2 = \mu_3 = \mu_a$. The null hypothesis was rejected where P <0.05 and a trend identified where 0.05< P > 0.1. Least squares means were used to calculate a pooled standard error of treatment means. Shapiro-Wilk tests were used to test for normality (Shapiro & Wilk, 1965).

6.3 Results

6.3.1 Trial A

A short summary of the production and rumen metabolism results of trial A are represented in Table 6-2. Treatment did not have an effect on milk yield; however, there was a linear decrease in 4% FCM yield as the level of DAP inclusion increased in the diet. Cows receiving the AP 0 concentrate supplement yielded 0.9 and 1.2 kg more 4% FCM than cows on both the AP 50 and AP 75 concentrate supplements (P <0.001), respectively. Treatment had no effect on the acetate to propionate ratio; however there was a linear trend for increasing acetate to propionate ratio as the level of DAP inclusion increased. Treatment had no effect on the NH_3-N concentration in the rumen. Furthermore, the mean ruminal pH measured over a 24 hour period showed a linear decrease from pH 6.23 for cows receiving the AP 0 concentrate supplement to pH 6.03 for cows receiving the AP 75 concentrate supplement (P <0.001). Treatment had no effect on the highest and lowest recorded pH.

Table 6-2 Summary of production and rumen metabolism results obtained for trial A.

Parameter[*]	Treatment[**]				SEM	Linear	Quadratic	Cubic
	AP 0	AP 25	AP 50	AP 75				
Production								
Milk yield (kg/d)	13.7	13.3	13.5	12.8	0.39	0.059	0.478	0.267
4% FCM yield (kg/d)	16.5[a]	16.3[ab]	15.6[bc]	15.3[c]	0.36	<0.001	0.804	0.438
Rumen metabolism								
Acetate: Propionate	3.32	3.49	3.66	3.62	0.13	0.059	0.410	0.688
NH_3-N (mM/L)	14.2	14.8	14.7	15.6	0.78	0.252	0.799	0.581
Mean pH	6.23[a]	6.21[a]	6.13[b]	6.03[c]	0.04	<0.001	0.040	0.590
Highest pH	6.48	6.47	6.59	6.65	0.18	0.545	0.858	0.855
Lowest pH	5.81	5.81	5.78	5.90	0.15	0.766	0.719	0.836

[a,b] Difference in superscript indicates significance at P <0.05.

[*] FCM-Fat corrected milk; NH_3-N-Ammonia nitrogen.

[**] AP 0: 0% dried apple pomace (DAP) inclusion; AP 25: 25% DAP inclusion; AP 50: 50% DAP inclusion; AP 75: 75% DAP inclusion.

The bacterial population of cows in trial A was made up of 16 different bacterial phyla (Figure 6-1). Of these 16, *Bacteroidetes*, *Firmicutes*, *Tenericutes* and *Proteobacteria* were the most prevalent, making up 92-98% of the total population. Treatment did not have an effect on the distribution of phyla in trial A except for *Tenericutes* and *Verrucomicrobia*, which were higher for cows in the AP 0 treatment compared to the other treatments; P = 0.001 and P = 0.043, respectively.

When the bacterial community was analysed at the family level, 139 families were identified, with 16 families each contributing >1% of the total sequences in any treatment (Figure 6-2). These top 16 families made up 84-92% of all the sequences read. The families *Acholeplasmataceae* and *Anaeroplasmataceae* were both affected by treatment, with the highest prevalence found in cows in the AP 75 treatment compared to cows in the AP 0, AP 25 and AP 50 treatments, P = 0.050 and P = 0.015, respectively. The prevalence of the *Bacteroidaceae* and *Erysipelotrichaceae* families all increased linearly as the level of DAP inclusion increased, whereas *Veillonellaceae* showed a linear trend for decreasing prevalence as the level of DAP inclusion increased.

A total of 47 genera were identified in trial A, with the 28 most prevalent genera making up 99.5-99.7% of the total bacterial population (Table 6-3). *Prevotella* was the most dominant genus, making up more than half of the bacterial community. The presence of *Prevotella* showed a linear trend, increasing as the level DAP increased (P = 0.079). The genera *Selenomonas* was the second most prevalent in the bacterial community with no treatment effect. *Treponema* was least prevalent for cows in the AD 0 treatment, with the population more than doubling for cows in the AD 75 treatment (P = 0.020). *Oribacterium* was highest for cows in the AD 25 treatment and lowest for cows in the AD 75 treatment (P = 0.034). *Anaerovibrio* showed a linear trend for decreasing prevalence as the level of DAP inclusion increased (P = 0.091). The genus *Anaeroplasma*, *Saccharofermentans*, *Catonella* and *Lachnospira* showed a linear trend, with prevalence increasing as the level of DAP increased; P = 0.048, P = 0.078, P = 0.046 and P = 0.082, respectively. The genera *Roseburia* and *Bulleidia* showed a quadratic trend with prevalence being highest for cows in the AD 25 and AD 50 treatments; P = 0.094 and P = 0.048, respectively.

Rarefaction analysis on species level indicated that bacterial diversity, or species richness, was similar between treatments, with AP 0 and AP 75 showing slightly lower diversity than AP 25 and AP 50 (Figure 6-3).
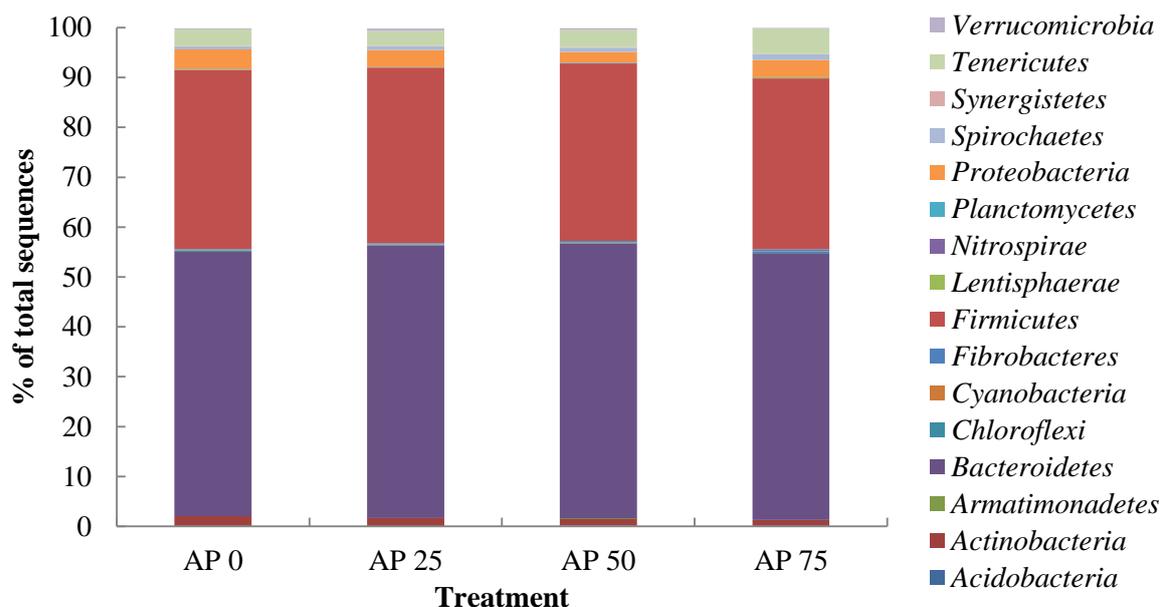
Figure 6-1 All phyla (as a % of the total bacterial population) of bacteria identified from the ruminal fluid of four cannulated cows receiving trial A diets. (AP 0: 0% dried apple pomace (DAP) inclusion; AP 25: 25% DAP inclusion; AP 50: 50% DAP inclusion; AP 75: 75% DAP inclusion)
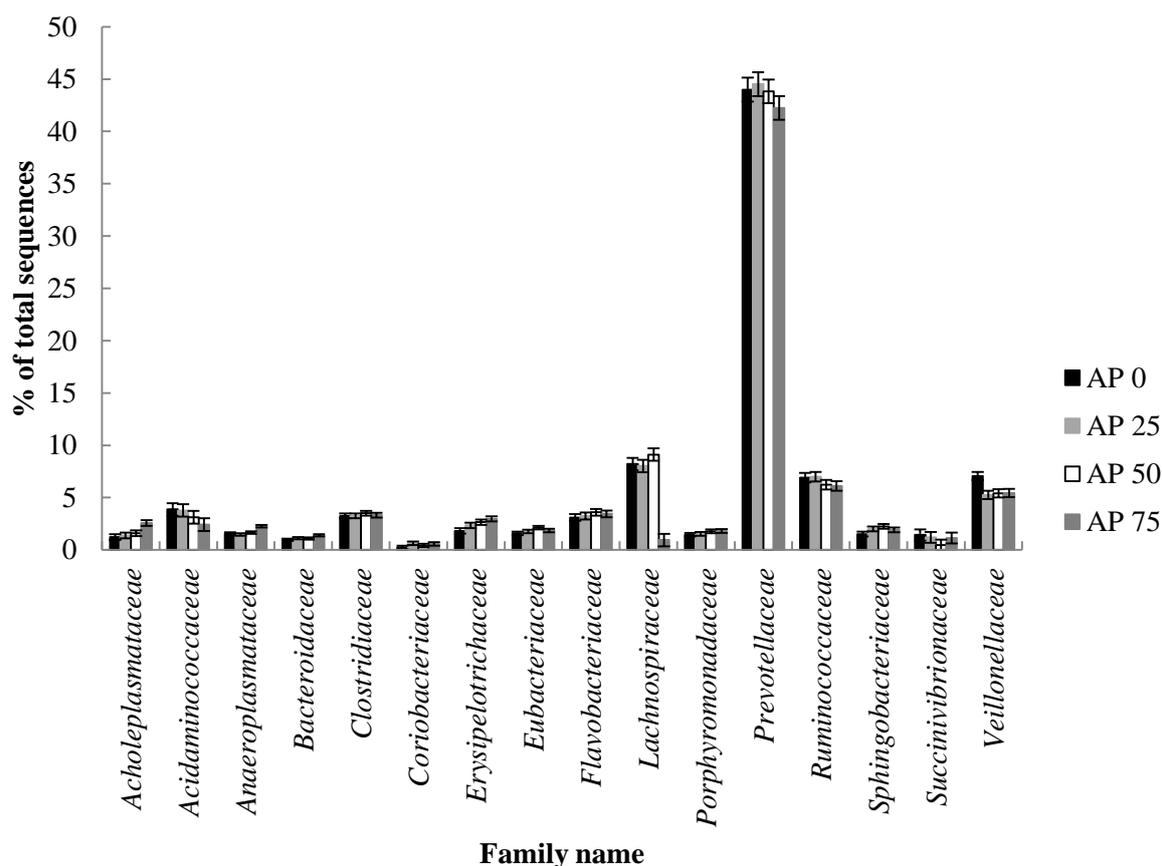
Figure 6-2 The top families as a % of the total bacterial population of bacteria identified from the ruminal fluid of four cannulated cows receiving trial A diets. (AP 0: 0% dried apple pomace (DAP) inclusion; AP 25: 25% DAP inclusion; AP 50: 50% DAP inclusion; AP 75: 75% DAP inclusion)

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Table 6-3 The most common genera (as a % of the total bacterial population) of bacteria identified from the ruminal fluid of four cannulated cows receiving trial A diets.

Parameter	Treatment*				SEM	Linear	Quadratic	Cubic
	AP 0	AP 25	AP 50	AP 75				
Prevotella	51.7	53.6	55.5	54.2	1.02	0.079	0.169	0.508
Selenomonas	10.5	8.42	9.79	8.46	0.87	0.258	0.370	0.164
Succiniclasticum	6.85	7.06	5.72	4.61	1.25	0.198	0.617	0.758
Ruminococcus	6.51	5.47	5.68	6.19	0.42	0.707	0.117	0.641
Anaerovibrio	6.09	4.99	4.17	4.76	0.53	0.091	0.164	0.649
Butyrivibrio	3.52	4.12	4.06	3.77	0.83	0.858	0.610	0.910
Ruminobacter	2.29	1.91	0.55	1.93	1.10	0.637	0.452	0.480
Lachnobacterium	1.51	1.53	1.78	1.49	0.22	0.840	0.505	0.463
Clostridium	1.30	1.48	1.78	1.40	0.27	0.634	0.336	0.529
Schwartzia	1.69	1.44	1.37	1.40	0.17	0.258	0.437	0.944
Coprococcus	1.13	1.15	1.41	1.59	0.21	0.134	0.724	0.760
Eubacterium	1.01	1.23	1.32	1.14	0.18	0.569	0.304	0.860
Treponema	0.56[a]	0.63[a]	1.00[ab]	1.39[b]	0.14	0.004	0.300	0.678
Olsenella	0.42	1.18	0.64	0.72	0.32	0.807	0.335	0.229
Anaeroplasma	0.50	0.80	0.70	0.92	0.10	0.048	0.723	0.175
Fibrobacter	0.44	0.51	0.51	1.30	0.36	0.160	0.351	0.615
Roseburia	0.53	0.79	0.83	0.46	0.10	0.740	0.020	0.670
Succinivibrio	0.65	0.56	0.60	0.63	0.15	0.986	0.723	0.819
Succinimonas	0.58	0.73	0.06	0.81	0.45	0.988	0.533	0.304
Faecalibacterium	0.44	0.50	0.63	0.51	0.07	0.300	0.258	0.354
Sutterella	0.25	0.26	0.26	0.54	0.15	0.239	0.381	0.661
Bifidobacterium	0.53	0.18	0.12	0.11	0.21	0.212	0.462	0.814
Saccharofermentans	0.15	0.22	0.24	0.32	0.06	0.078	0.967	0.670
Catonella	0.09	0.26	0.23	0.33	0.06	0.046	0.584	0.296
Bulleidia	0.16	0.24	0.18	0.07	0.04	0.129	0.048	0.640
Oribacterium	0.18[ab]	0.26[a]	0.12[b]	0.07[b]	0.03	0.024	0.111	0.073
Atopobium	0.06	0.18	0.14	0.22	0.07	0.235	0.759	0.458
Lachnospira	0.04	0.05	0.19	0.23	0.08	0.082	0.831	0.519
Total	99.6	99.7	99.5	99.6				

[a,b] Difference in superscript indicates significance at P <0.05.
* AP 0: 0% dried apple pomace (DAP) inclusion; AP 25: 25% DAP inclusion; AP 50: 50% DAP inclusion; AP 75: 75% DAP inclusion.
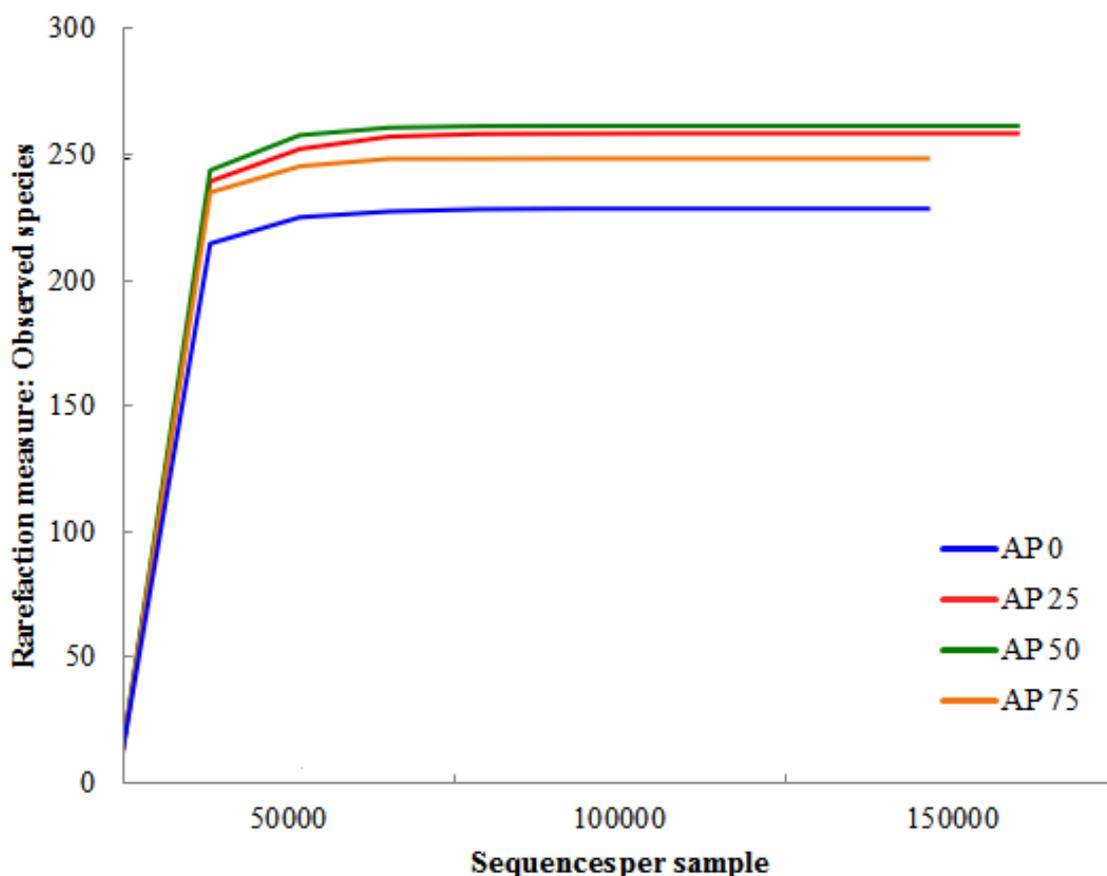
Figure 6-3 Rarefaction analysis of observed species of rumen bacterial species identified from the ruminal fluid of four cannulated cows receiving trial A diets. (AP 0: 0% dried apple pomace (DAP) inclusion; AP 25: 25% DAP inclusion; AP 50: 50% DAP inclusion; AP 75: 75% DAP inclusion)

6.3.2 Trial B

A summary of the production and rumen metabolism results that were observed in trial B are presented in Table 6-4. Milk yield decreased linearly as the level of DAP inclusion increased. The 4% FCM yield was lower for cows in treatment HDAP than for cows in treatments NDAP and LDAP and for cows in treatment MDAP compared to cows in treatment NDAP (P <0.001), with differences ranging between 1.7 and 2.3 kg/day. The acetate to propionate ratio and the NH_3-N concentration did not differ between treatments. Furthermore, the mean pH over a 24 h period and the highest pH recorded did not show any treatment effects. The lowest pH recorded showed a quadratic trend, with the lowest pH being recorded for the NDAP treatment.

Table 6-4 Summary of production and rumen metabolism results obtained for trial B.

Parameter[*]	Treatment[**]				SEM	Linear	Quadratic	Cubic
	NDAP	LDAP	MDAP	HDAP				
Production								
Milk yield (kg/d)	19.1	18.3	18.6	17.4	0.63	0.028	0.762	0.227
4% FCM yield (kg/d)	20.9[a]	20.5[ab]	19.2[bc]	18.6[c]	0.64	<0.001	0.783	0.485
Rumen metabolism								
Acetate: Propionate	3.28	3.34	3.30	3.34	0.18	0.865	0.941	0.813
NH_3-N (mM/L)	9.13	9.49	10.8	10.0	0.74	0.231	0.327	0.257
Mean pH	6.09	6.37	6.30	6.18	0.13	0.730	0.157	0.641
Highest pH	6.55	6.83	6.78	6.69	0.17	0.648	0.318	0.708
Lowest pH	5.68	6.02	5.86	5.77	0.09	0.780	0.049	0.209

[a,b] Difference in superscript indicates significance at $P < 0.05$.

[*] FCM-Fat corrected milk; NH_3-N-Ammonia nitrogen.

[**] NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP.

The total bacterial population of cows in trial B was made up of 16 different bacterial phyla (Figure 6-4). *Bacteroidetes*, *Firmicutes*, *Tenericutes* and *Proteobacteria* were the most prevalent here and made up 92-98% of the total population. Only the *Proteobacteria* were affected by treatment, with the population decreasing as the level of DAP inclusion increased ($P = 0.041$). Otherwise, no treatment effect was seen on the distribution of the bacterial phyla in the population.

When the bacterial community was analysed at the family level, 145 families could be identified, with 16 families each contributing >1% of the total sequences in any treatment (Figure 6-4). These top 16 families made up 84-95% of the total population. Of these 16 different families *Porphyromonadaceae* increased linearly as the level of DAP inclusion increased ($P = 0.019$) and *Ruminococcaceae* showed a decreasing linear trend in abundance when DAP was included ($P = 0.028$). *Succinivibrionaceae* was highest for cows in the NDAP treatment compared to cows in the LDAP, MDAP and HDAP treatments ($P = 0.005$). *Veillonellaceae* was highest for cows in the MDAP and HDAP treatments and lowest for cows in the NDPA treatment ($P = 0.013$).

A total of 61 genera were identified in trial B, with 32 genera making up 99.4-99.8% of the total bacterial community (Table 6-5). *Prevotella* was the most dominant genus, making up 44-54.4% of the total population. The genera *Prevotella*, *Selenomonas* and *Catonella* all showed a linear trend with the population increasing as the level of DAP increased; $P = 0.071$, $P = 0.057$ and $P = 0.037$, respectively. The genera *Succinimonas*, *Kandleria* and *Oribacterium* also showed linear trends; however, their prevalence in the population decreased as the level DAP inclusion increased; $P = 0.024$, $P = 0.051$ and $P = 0.089$, respectively. *Eubacterium* and *Anaeroplasma* showed a cubic trend in response to DAP inclusion; $P = 0.094$ and $P = 0.064$, respectively. The population of *Ruminobacter* was highest for cows in treatment NDAP,

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decreasing to 0% for cows in treatment HDAP (P = 0.024). The population of *Treponema* was highest for cows in treatment HDAP and lowest for cows in treatment MDAP (P = 0.037).

Rarefaction analysis on species level indicated that bacterial diversity, or species richness, was similar between treatments, except for treatment HDAP showing lower diversity than NDAP, LDAP and MDAP (Figure 6-6).
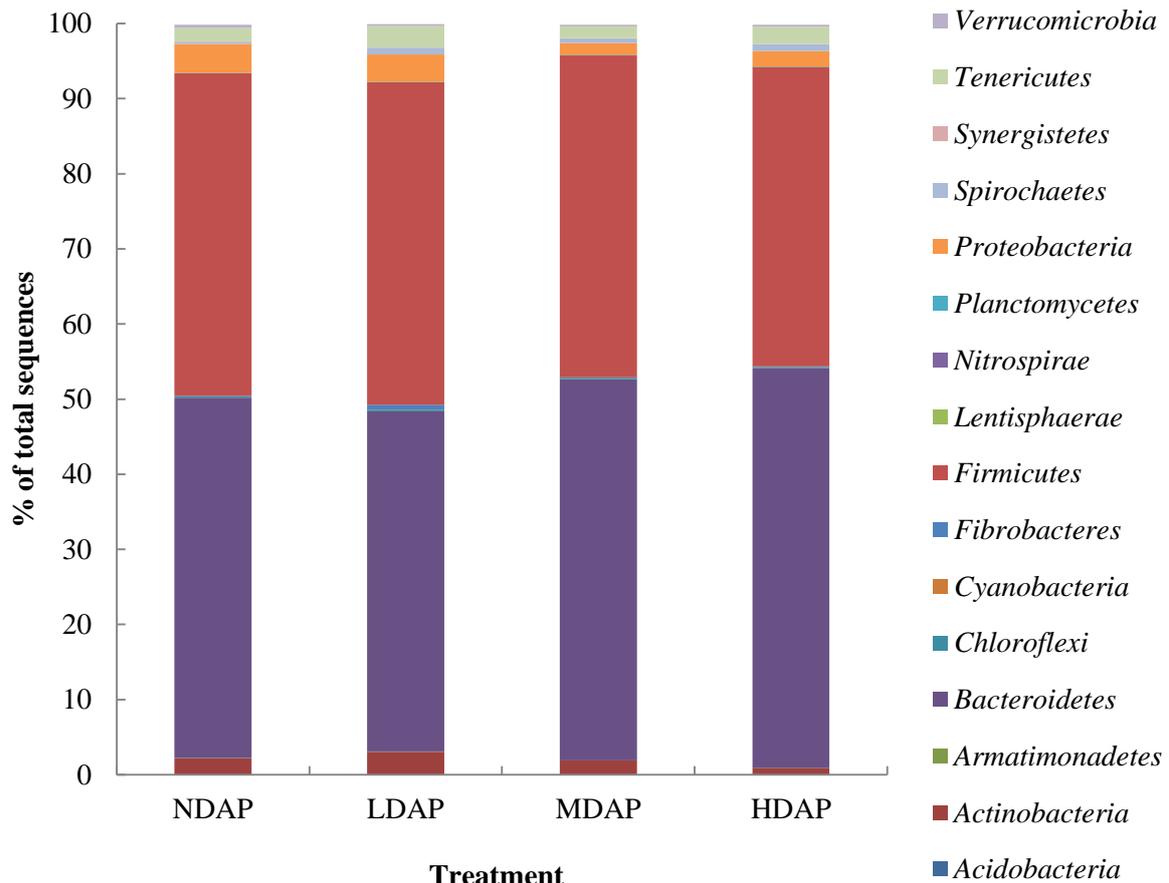
Figure 6-4 All phylum (as a % of the total bacterial population) of bacteria identified from the ruminal fluid of four cannulated cows receiving trial B diets. (NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP)
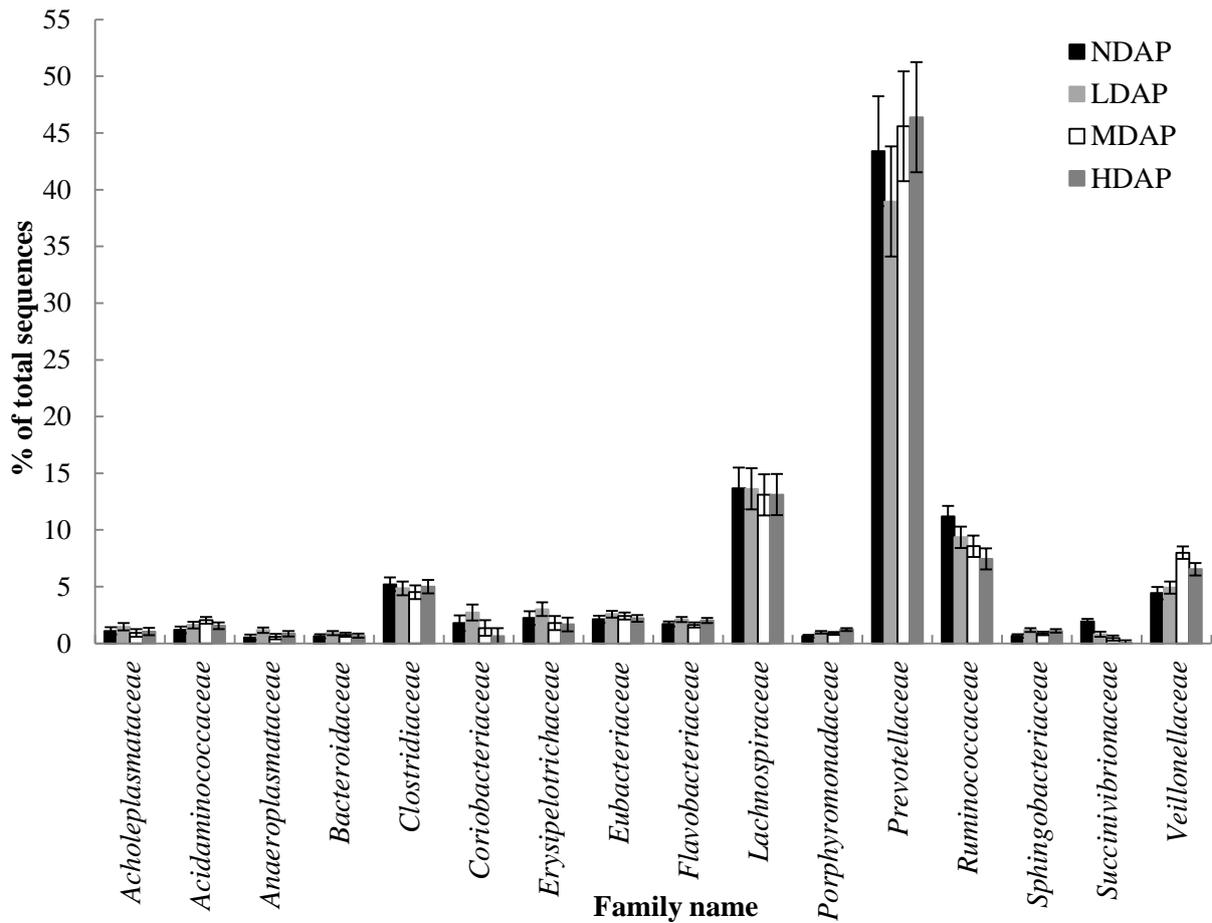
Figure 6-5 The top families represented as a % of the total bacterial population of bacteria identified from the ruminal fluid of four cannulated cows receiving trial B diets. (NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP)

Table 6-5 The most common genera (as a % of the total bacterial population) of bacteria identified from the ruminal fluid of four cannulated cows receiving trial B diets.

Parameter	Treatment[*]				SEM	Linear	Quadratic	Cubic
	NDAP	LDAP	MDAP	HDAP				
Prevotella	44.0	46.2	47.9	54.4	3.36	0.071	0.540	0.735
Ruminococcus	14.8	9.61	6.73	9.11	2.51	0.124	0.180	0.803
Selenomonas	6.47	6.91	12.9	9.32	1.39	0.057	0.197	0.051
Butyrivibrio	7.81	7.40	7.09	6.78	1.92	0.706	0.981	0.991
Anaerovibrio	2.35	3.35	4.72	4.40	0.78	0.074	0.430	0.575
Succiniclasticum	1.97	2.63	3.19	2.94	0.41	0.107	0.307	0.718
Olsenella	2.93	4.39	1.99	0.96	1.10	0.142	0.301	0.328
Eubacterium	1.98	1.85	2.52	1.33	0.30	0.381	0.125	0.094
Lachnobacterium	1.64	2.08	1.84	1.41	0.36	0.587	0.278	0.770
Ruminobacter	4.89[a]	1.35[b]	0.67[b]	0.00[b]	0.02	0.007	0.138	0.479
Schwartzia	1.18	1.78	1.45	1.30	0.39	0.988	0.364	0.541
Clostridium	1.26	1.35	1.40	1.66	0.25	0.307	0.743	0.813
Faecalibacterium	0.90	1.54	1.42	1.22	0.22	0.417	0.101	0.494
Coprococcus	1.21	1.15	1.33	1.29	0.24	0.719	0.968	0.686
Roseburia	0.77	0.78	1.04	0.78	0.20	0.770	0.527	0.436
Atopobium	0.52	1.12	0.48	0.20	0.35	0.351	0.257	0.358
Fibrobacter	0.21	1.74	0.05	0.23	0.82	0.672	0.440	0.214
Treponema	0.37[ab]	0.62[bc]	0.25[a]	0.78[c]	0.10	0.109	0.212	0.016
Acinetobacter	1.53	0.05	0.02	0.17	0.66	0.217	0.265	0.685
Sutterella	0.16	0.78	0.16	0.05	0.34	0.547	0.324	0.291
Anaeroplasma	0.18	0.38	0.19	0.33	0.07	0.456	0.651	0.064
Lachnospira	0.10	0.10	0.80	0.10	0.30	0.631	0.285	0.162
Succinimonas	0.39	0.30	0.26	0.00	0.09	0.024	0.367	0.541
Kandleria	0.37	0.31	0.08	0.06	0.12	0.051	0.834	0.460
Bulleidia	0.29	0.26	0.12	0.13	0.07	0.107	0.804	0.448
Saccharofermentans	0.16	0.20	0.18	0.18	0.05	0.869	0.643	0.702
Oribacterium	0.22	0.21	0.19	0.09	0.05	0.089	0.373	0.769
Catonella	0.09	0.15	0.22	0.24	0.04	0.037	0.680	0.703
Pseudobutyrivibrio	0.14	0.31	0.17	0.07	0.09	0.402	0.179	0.427
Succinivibrio	0.18	0.25	0.15	0.10	0.05	0.213	0.261	0.387
Blautia	0.18	0.16	0.10	0.11	0.04	0.170	0.774	0.542
Pseudomonas	0.19	0.15	0.08	0.06	0.10	0.335	0.924	0.856
Total	99.4	99.5	99.7	99.8				

[a,b] Difference in superscript indicates significance at P <0.05.
[*] NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP.
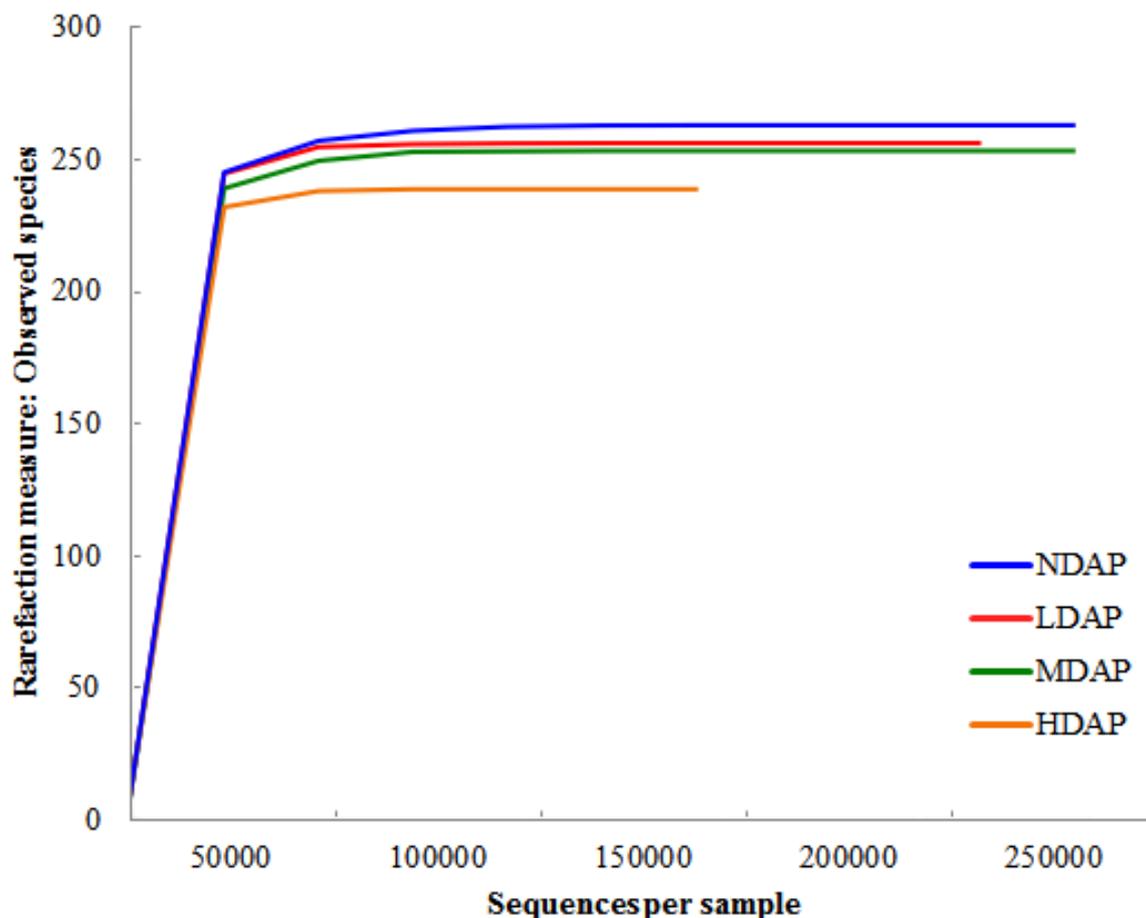
Figure 6-6 Rarefaction analysis of observed species of rumen bacterial species identified from the ruminal fluid of four cannulated cows receiving trial B diets. (NDAP-0% dried apple pomace (DAP); LDAP-25% DAP; MDAP-50% DAP; HDAP-75% DAP)

6.4 Discussion

There was a definite decrease in starch and increase in sugar and NDF content of the four different diets of each trial as the level of DAP inclusion increased. This also translated into the production results where milk yield and 4% FCM yield were influenced by treatment. There was almost a complete lack of response in rumen metabolism results, posing the question, what caused the response in production but did not influence rumen metabolism? It has been observed by Palmonari *et al.* (2010) and Welkie *et al.* (2010) that changes in ruminal bacterial community dynamics between various treatments do not necessarily translate into different rumen metabolism outputs. This was confirmed in the current investigation, where various changes in the bacterial community on family and genus level were observed, even though factors such as ruminal pH, NH_3-N and VFA concentration did not show a response to treatment or at least showed a minimal response.

In both trial A and B the phyla *Bacteroidetes* and *Firmicutes* were the most prevalent, which agrees with previous research. In a study by de Menezes *et al.* (2011) the ruminal bacterial community dynamics were compared between cows grazing pasture and cows receiving a TMR based diet. They detected 14 phyla overall, of which *Bacteroidetes* and *Firmicutes* represented around 80% of the total sequences and there was no difference between treatments. In trial A and B of the current study, the *Bacteroidetes* and *Firmicutes* represented 88-91% and 88-94%, respectively, this is a bit higher than found by de Menezes *et al.* (2011). However, Broadway *et al.* (2012) found 22 bacterial phyla in beef heifers fed a TMR with varying levels of citrus pulp pellets, with *Bacteroidetes* and *Firmicutes* making up 91% of the bacterial community. The ratio of *Firmicutes* to *Bacteroidetes* is also used to expand on the ruminal bacterial community dynamics; however, no treatment effects were observed on this variable in either of trial A or trial B. In a study of Callaway *et al.* (2010) the effect of including dried distillers grains in the diet fed to steers on bacterial diversity was determined. It was found that as the level of dried distiller's grains increased the ratio of *Firmicutes* to *Bacteroidetes* decreased; the pH of the diet is said to have played a big role here. Fernando *et al.* (2010) investigated how the rumen bacteria changed in steers adapting to high maize grain diets. It was found that as the level of grain inclusion in the diet increased the rumen pH decreased and the ratio of *Firmicutes* to *Bacteroidetes* decreased, similar to Callaway *et al.* (2010). In both trial A and B of the current study there was no response of rumen pH to treatment and the *Firmicutes* to *Bacteroidetes* ratio was not affected. Deductions from previous research would suggest that a lower ruminal pH could decrease the *Firmicutes* to *Bacteroidetes* ratio. An iincrease in ruminal pH was expected in both trial A and B, which could then have led to an increase in the *Firmicutes* to *Bacteroidetes* ratio. The *Firmicutes* to *Bacteroidetes* ratio plays an important role in milk production and milk yield efficiency and even possibly on residual feed intake (Guan *et al.*, 2008; Jewell *et al.*, 2015). As such, an improvement in ruminal pH could lead to higher *Firmicutes* to *Bacteroidetes* ratio, possibly increasing production output.

More variation and treatment effects were seen in the family data for both trial A and B. The most prevalent families detected were *Prevotellaceae*, *Lachnospiraceae*, *Veillonellaceae*, *Ruminococcaceae* and *Clostridiaceae*, which is similar to the findings of de Menezes *et al.* (2011). When the bacterial community dynamics were compared between cows grazing pasture and cows receiving TMR, it was found that *Prevotellaceae*, *Erysipelotrichaceae* and *Lachnospiraceae* prevalence were higher for cows grazing pasture, which correspond to trial A and B. Jewell *et al.* (2015) conducted an in depth study of ruminal bacterial community

dynamics of cows across lactations. Four family groups, namely *Acidaminococcaceae*, *Lachnospiraceae*, *Prevotellaceae* and *Ruminococcaceae*, were identified as highly conserved, 'core bacterial families' in the dairy cow rumen. de Menezes *et al.* (2011) found that the abundance of *Fibrobacteraceae* increased for cows fed a TMR ration, which is interesting to note as *Fibrobacteraceae* had a very low abundance in both trial A and B (<0.24%). On the other hand *Erysipelotrichaceae* was more abundant in cows fed pasture (de Menezes *et al.*, 2011) and was one of the most prevalent families in both trial A and B. In two parallel trials by de Menezes *et al.* (2011) and O'Neill *et al.* (2011) a 60% decrease in methane gas production was observed for cows grazing pasture, with these cows also having a higher abundance of *Prevotellaceae*, *Erysipelotrichaceae* and *Veillonellaceae*. Little is known about the metabolism of *Erysipelotrichaceae*; however, it is known that members of both *Prevotellaceae* and *Veillonellaceae* produce propionate as a major fermentation product (Strobel & Russel, 1991), which contributes to reduced methanogenesis due to diversion of H-molecules (Janssen, 2010 and Ungerfeld, 2015). In previous research where the effect of carbohydrate source on methanogenesis was investigated no clear response was found, with higher pectin and sugar content and lower starch content increasing and decreasing methanogenesis at times (Hindrichsen *et al.*, 2005 and Poulsen *et al.*, 2012). However, researchers in both studies emphasised the importance of ruminal pH in regulating methanogenesis. It could be deduced that an increase in ruminal pH, as with cows on pasture based systems compared to cows on TMR systems, could stimulate the abundance of families such as *Veillonellaceae*, leading to lowered methanogenesis.

The profile of the most prevalent genus differed between trial A and B. It is interesting to note that only eight of the top 16 genera identified by Callaway *et al.* (2010) were present in the most prevalent genera here. Callaway *et al.* (2010) used steers on feedlot rations, so the difference in the genus profile is not surprising. Furthermore, Fernando *et al.* (2010) found that steers fed forage based diets had higher ruminal bacterial diversity than steers fed high grain diets, confirming the high genera diversity found in the current study. In general, similar genera are the most prevalent in various trials, with the order of abundance differing slightly. Broadway *et al.* (2012) found the five genera *Prevotella*, *Eubacterium*, *Ruminococcus*, *Clostridium* and *Roseburia* to be the most abundant, all these genera were also present in the top 15-17 genera identified in trial A and B. *Prevotella* was the most abundant genera in both trial A and B, across all treatments. In a study by Jewell *et al.* (2015) *Prevotella* was associated with both high and low efficiencies of milk production and is classically considered beneficial

to rumen function. Interestingly, in a study by Pitta *et al.* (2010) the abundance of *Prevotella* increased when steers were moved from bermudagrass hay to a wheat pasture. They also identified a synergistic association between *Prevotella* and *Treponema* and that these genera were related to rapid degradation of fibre and soluble fractions. In the current study, the abundance of *Prevotella* increased linearly as DAP inclusion increased for both trial A and B. Furthermore, *Treponema* prevalence was highest when DAP was supplemented for ground maize in the AP 75 trial A treatment and the LDAP and HDAP trial B treatments. This increase in prevalence of *Prevotella* and *Treponema* was probably in response to higher NDF levels in the diet as well as higher sugar content. The genus *Succiniclasticum* was found in cows in all treatments of both trials. *Succiniclasticum* is characterised by its absolute dependence on succinate as a substrate converted to propionate (van Gylswyk, 1995), which also corresponds to the lower methanogenesis recorded for cows grazing pasture compared to cows fed TMR (Buddle *et al.*, 2011).

The usefulness of rarefaction curves as an indication of species richness is debatable; however, applying the same principles across treatments may still provide a usable answer (Hughes *et al.*, 2001). Furthermore, the plateau that is reached at around 50 000 reads for all of treatments of trial A and B is indicative thereof that close to all possible species were identified (Hughes *et al.*, 2001). The question of sample number is also important to consider, as species richness is influenced by how representative a sample is (Guan *et al.*, 2008). In ruminal bacterial community dynamics work done previously, sampling times, methods and the number of cows sampled all varied according to the resources that were available. Essentially, more samples per animal, collected over days would provide more depth of knowledge; however, fewer samples do not invalidate the results found. Considering the ruminal bacterial community composition includes an in depth look at individual species as well. However, when ruminal bacterial community dynamics are considered, a more general overview is given, looking in depth at the phyla, genera and families present and how they respond to an experimental treatment. Further analyses will be done to consider individual bacterial species communities; however, it is outside of the scope of this paper.

6.5 Conclusion

There was an observable difference in in the bacterial community dynamics between cows grazing kikuyu pasture in trial A compared to cows grazing ryegrass pasture in trial B. There were significant bacterial community shifts between feeding higher starch and low fibre

concentrates compared to high sugar and high fibre concentres, on both kikuyu and ryegrass pastures. This shift in the ruminal bacterial community did not translate into rumen metabolism differences; however, differences in cow production and efficiency were influenced by treatment. The study did not provide clear clarification for the production results found; however, it can be concluded that feeding high levels of DAP to cows grazing kikuyu or ryegrass pasture was not detrimental to the ruminal bacterial community. Future studies should focus on the bacterial community of rumen solids and liquids separately, as well as consider other ruminal micro-organisms, especially Archaea.

6.6 References

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Chapter 7: Conclusion and recommendations

Dairy production from kikuyu-ryegrass pastures is a widely used and well understood system in the southern Cape of South Africa. Throughout the year additional supplementation, mostly in the form of a concentrate supplement, is also provided. Improvement of this system can be approached from a pasture, supplemental feeding and management angle, with the most immediate response visible through manipulation of the supplemental feeding. Supplemental feeding is also one of the biggest expenses in such a system and as such any improvement in input costs and outputs obtained will have a large impact on the success of a dairy farm. When the current world population growth and climate change is considered, it is of utmost importance to investigate alternative feed sources, which are often regarded as waste products, and their place in the animal feed industry. Fruit waste is one such product, with citrus pulp and apple pomace being two of the most readily available fruit wastes in South Africa.

Three production trials were carried out to determine the effectiveness of replacing ground maize as the main energy source with dried citrus pulp (DCP) and dried apple pomace (DAP) for Jersey cows grazing kikuyu over sown with ryegrass pasture. Production parameters, such as milk yield, milk composition and BCS of cows, rumen metabolism parameters, such as pasture degradability, ruminal pH and VFA concentration, and pasture and energy intake parameters were investigated. The ME and CP content of DCP and DAP varies more than that of maize, and there is a chance of purchasing a low quality DCP or DAP (with regards to ME and CP content). However, in all three trials the ME obtained from concentrates with varying levels of ground maize and DCP or DAP were similar between treatments. Treatment specific pasture DMI was also determined for the two DAP trials. On kikuyu pasture the replacement of ground maize with DAP did not influence pasture DMI; however, on ryegrass pasture replacing 100% of ground maize with DAP resulted in lower pasture DMI. The response of milk yield and 4% FCM yield were similar between the three trials, with both milk yield and 4% FCM yield decreasing linearly as the level of ground maize replacement increased. On the two trials where cows grazed ryegrass pasture 4% FCM yield decreased 3.1 kg/cow and 2.3 kg/cow when ground maize was replaced 100% with DCP and DAP, respectively. When ground maize was replaced 100% with DAP for cows grazing kikuyu pasture 4% FCM yield only decreased by 1.2 kg/cow. The effect of DCP and DAP on milk composition was more variable across trials, with only the replacement of ground maize with DAP on the kikuyu pasture not negatively affecting milk fat and milk protein content. On ryegrass pasture, milk

fat content remained unchanged but milk protein content responded quadratically and milk fat and milk protein content decreased linearly when ground maize was replaced with DCP or DAP, respectively. Across all three trials treatment did not have an effect on BW or BCS change. The rumen metabolism also remained mostly unaffected by treatment across all three trials. The mean rumen pH was only affected by the level of ground maize replaced with DAP for cows grazing kikuyu pasture, but the mean rumen pH was still above pH 6 and would not be expected to negatively impact the rumen environment. Treatments across all three trials did not influence VFA concentration and only cows on kikuyu pasture showed a linear increase in the acetate to propionate ratio as the level of DAP inclusion increased. No improvement in pasture DMD and NDFD were observed when ground maize was replaced with DCP or DAP on kikuyu and ryegrass pasture. Ruminal bacterial community dynamics were influenced by the replacement of ground maize with DAP on kikuyu and ryegrass pasture; however, no negative populations shifts were found.

Recommendations for the use of DCP and DAP as an energy source for cows grazing kikuyu and ryegrass pasture will depend on availability and cost, as well as the current state of the dairy industry. The use of both DCP and DAP on kikuyu and ryegrass pasture did not have a negative effect on the rumen environment compared to feeding ground maize as the main energy source. Furthermore, pasture DMI and pasture degradability remained mostly unaffected by DCP and DAP inclusion, even though the literature would suggest that improvements could be expected. Milk yield and 4% FCM yield were negatively affected by the 100% replacement of ground maize with DCP and DAP; however, the effect thereof must be weighed against the financial gain of a more affordable feed source as well as the gain of reserved human food, not fed to animals. As such, the level at which maize could be replaced should be weighed against the potential loss in milk production and the potential decrease in feed price. In situations of drought, when the maize price tends to increase, or when the milk price is very low, perhaps due to an over production of milk in an area, it would be sensible to include a more affordable feed source such as DCP or DAP, as the financial loss of lowered milk production could be outweighed by the lower feed cost.

The importance of fruit wastes, such as DCP and DAP, as an animal feed should not be over-looked, especially on pasture based dairy systems where it has been shown as a viable feed option for incorporation into a concentrate supplement. The level of maize replacement should be considered carefully, but from this study only partial replacement (33-66 %) of ground maize with DCP or DAP would be recommended.

Chapter 8: Appendix

All equations to linear, quadratic and cubic trends observed for production and rumen metabolism data, including R^2 coefficients. Variable x refers to the amount of DCP or DAP included (kg DM/day) in the concentrate supplement. Equations are in order of appearance in relevant chapter.

8.1 Chapter 3: Replacing maize grain with dried citrus pulp in a concentrate feed for Jersey cows grazing ryegrass pasture

- Milk yield (kg/cow) = 20.68 - 0.6467x (R^2 = 0.867)

- 4% FCM (kg/cow) = 21.99 - 0.64x (R^2 = 0.839)

- Protein content (g/kg) = 34.905 + 0.9367x - 0.2333x^2 (R^2 = 0.999)

- Lactose content (g/kg) = 46.455 + 0.77x - 0.2111x^2 (R^2 = 0.969)

- MUN content (mg/dL) = 9.286 + 0.2173x (R^2 = 0.714)

- Fat yield (kg/cow) = 0.919 - 0.0273x (R^2 = 0.851)

- Protein yield (kg/cow) = 0.728 - 0.0247x (R^2 = 0.941)

8.2 Chapter 4: The effect of replacing maize with dried apple pomace in the concentrate on performance of Jersey cows grazing kikuyu pasture

- Milk yield (kg/cow) = 13.7 - 0.1667x (R^2 = 0.698)

- 4% FCM (kg/cow) = 16.57 - 0.2867x (R^2 = 0.956)

- Fat yield (kg/cow) = 0.735 - 0.0133x (R^2 = 0.800)

- Protein yield (kg/cow) = 0.519 - 0.004x (R^2 = 0.900)

- Lactose content (g/kg) = 44.34 + 0.3267x (R^2 = 0.786)

- NDF intake as % BW (kg DM) = 1.111 + 0.0407x (R^2 = 0.584)

- Energy in milk (MJ/day) = 51.71 - 0.9267x (R^2 = 0.987)

- Mean pH = 6.252 - 0.0453x (R^2 = 0.932)

8.3 Chapter 5: The effect of dried apple pomace as a replacer for maize in the concentrate for Jersey cows grazing ryegrass pasture on production and rumen metabolism

- Pasture DMI (kg/day) = 12.598 - 0.6647x (R^2 = 0.742)

- Total DMI (kg/day) = 18.03 - 0.6467x (R^2 = 0.727)

- DMI as % BW (kg DM) = 4.578 - 0.1313x (R^2 = 0.692)

- Milk yield (kg/day) = 19.07 - 0.32x (R^2 = 0.753)

- 4% FCM (kg/day) = 21.03 - 0.5467x (R^2 = 0.961)

- Fat yield (kg/day) = 0.891 - 0.026x (R^2 = 0.838)

- Protein yield (kg/day) = 0.688 - 0.0113x (R^2 = 0.863)

- Fat content (g/kg) = 46.94 - 0.64x (R^2 = 0.296)

- Lactose content (g/kg) = 46.7 + 0.2333x (R^2 = 0.660)

- MUN content (mg/dL) = 11.69 + 0.26x (R^2 = 0.600)

- Total ME intake (MJ/cow) = 179.4 - 6.4x (R^2 = 0.698)

- Energy in milk (MJ/day) = 68.82 - 2.1533x (R^2 = 0.824)

- Time spent below pH 5.8 (hours) = 6.7235 - 2.811x + 0.7078x^2 (R^2 = 0.9853)