Quantification and mitigation of eneteric methane emissions from grazing jersey cows

by

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SUMMARY

QUANTIFICATION AND MITIGATION OF ENTERIC METHANE EMISSIONS FROM GRAZING JERSEY COWS

by

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Increasing evidence for global warming has amplified the need to accurately verify national greenhouse gas (GHG) inventories, and to validate on-farm GHG mitigation strategies. Agriculture is known to significantly contribute to GHG with ruminants identified as the single most important source of anthropogenic methane (CH_4) emissions – a potent GHG. Several CH_4 mitigation strategies have been developed, but few succeeded in terms of instant results, efficacy, persistency and practicality, of which all are vital for the adoptability thereof on farm level. Concentrate feeding level and dietary nitrate addition have been identified as CH_4 mitigation strategies that are most likely to be adopted on farm level. Although the latter CH_4 mitigation strategies have been extensively evaluated on cattle in confinement, limited research exist on the effect thereof on pasture-based dairy cattle across seasons.

This study aimed to directly measure CH_4 emissions from pasture-based Jersey cows grazing pasture, while determining the effect of concentrate feeding level and dietary nitrate addition as CH_4 mitigation strategies on CH_4 emissions, cow performance and rumen fermentation. Additionally, this study aimed to reduce or eliminate animal skin abrasions imposed by current back-mounted harnesses facilitating the SF_6 technique, and

to compare CH_4 data derived from the SF_6 technique with that of a short-term measurement technique. This study consisted of six trials.

The first trial investigated the effect of concentrate feeding level (0, 4, and 8 kg/cow per day; as fed basis) on enteric CH_4 emissions, cow production performance and rumen fermentation of dairy cows grazing kikuyu (*Pennisetum clandestinum*) dominant summer pasture. Sixty multiparous Jersey cows (nine rumen-cannulated) were used in a randomised complete block design with the cannulated cows in a 3 × 3 Latin square design. Total dry matter intake (DMI), milk yield, energy-corrected milk (ECM), and milk lactose content increased linearly, while pasture DMI decreased linearly with increasing concentrate feeding level. Methane production (323 to 378 g/d) increased linearly, while CH_4 yield and CH_4 intensity (milk yield and ECM) decreased linearly with increasing concentrate feeding level. Diurnal ruminal pH and *in sacco* dry matter (DM) and neutral detergent fibre (NDF) disappearance decreased linearly with increasing concentrate feeding level. Accetic and propionic acid were unaffected by treatment, while butyric acid increased linearly and quadratically with increasing concentrate feeding level. It was concluded that supplementing a high concentrate feeding level on pasture-only diets increases enteric CH_4 production by 17% but reduces CH_4 emissions per unit of DMI by 14% and per unit of milk yield and ECM by 39% and 41%, respectively, from cows grazing kikuyu-dominant pasture during summer.

The second trial was in essence a repeat of the first trial, but on ryegrass (*Lolium perenne*) dominant pasture to determine whether the CH_4 mitigation efficacy of the concentrate feeding level was influenced by seasonal change in pasture composition. Sixty multiparous Jersey cows (six rumen-cannulated) were used. Total DMI, milk yield, ECM, milk lactose content and pasture DMI response were similar to the previous trial, however in this trial milk fat content decreased with increasing concentrate feeding level. Volatile fatty acid concentrations and ruminal pH were mostly unaffected by treatment, while DM disappearance decreased and NH_3-N concentration increased with increasing concentrate feeding level. Methane production (258 to 302 g/d) and CH_4 yield were unchanged, while CH_4 intensity decreased linearly with increasing concentrate feeding level. It was concluded that concentrate supplementation on high quality pasture-only diets have the potential to effectively reduce CH_4 emissions per unit of milk yield by 20% from cows grazing perennial ryegrass-dominant pasture during spring.

The third trial investigated the effect of dietary nitrate addition (0, 11, and 23 g of nitrate/kg of DM; control, low nitrate, and high nitrate, respectively) on enteric CH_4

emissions, cow production performance and rumen fermentation of dairy cows grazing kikuyu-dominant pasture containing approximately 3 g of nitrate/kg of DM. Fifty-four multiparous Jersey cows (six rumen-cannulated cows) were used in a randomised complete block design with the cannulated cows in a 3 × 3 Latin square design. Concentrate was fed at 5.4 kg of DM/cow per d and formulated to be isonitrogenous by substituting urea. Cows were gradually adapted to concentrates over a 3-wk period. Although total DMI was unchanged, the high nitrate diet decreased concentrate DMI and milk yield but increased pasture DMI. Daily CH_4 production (313 to 280 g/d), CH_4 yield (21.8 to 18.7 g/kg of DMI) and CH_4 energy per gross energy intake (Y*m*; 6.9 to 5.9%) tended to decrease linearly with increasing dietary nitrate addition. It was concluded that dietary nitrate fed to grazing dairy cows showed some promise as CH_4 mitigation strategy. Furthermore, rumen fermentation was not adversely affected; however when feeding high levels of nitrate a decrease in milk yield could be expected due to a decrease in concentrate DMI.

The fourth trial was a repeat of the third trial, but on perennial ryegrass-dominant pasture and with only two treatments with essentially a different experimental design. The high nitrate treatment in the third trial was not repeated due to the observed partial refusal of concentrate and decreased milk yield. Thirty-two intact and eight rumen-cannulated multiparous Jersey cows were subjected to a replicated 2 × 2 Latin square design supplemented with one of two concentrates containing either urea (urea treatment), or urea and nitrate (nitrate treatment; containing 0.3 and 15.2 g of nitrate/kg of DM, respectively). Grazed pasture contained approximately 7.3 g of nitrate/kg of DM. Total DMI, milk yield, CH_4 production (400 and 405 g/d) and most rumen fermentation parameters were unaffected by treatment. Dietary nitrate increased milk components (except for milk protein content). Minor effects on ruminal pH were observed with an increasing tendency towards the nitrate group. It was concluded that although dietary nitrate supplementation is not an effective CH_4 mitigation strategy for dairy cows grazing perennial ryegrass, increases in milk fat and lactose content may be expected.

The fifth trial focused on improving the back-mounted harness of the SF_6 technique throughout the four main trials in terms of minimising skin abrasions and lesions imposed by the harness. In conclusion, a cost-effective back-mounted harness for grazing dairy cows that facilitates the SF_6 technique for measurement of enteric methane emissions while not causing any skin lesions was developed.

The sixth trial was a comparison study between CH_4 emission rates as measured by the LMD and SF_6 technique from lactating dairy cows grazing perennial ryegrass-

dominant pasture. Methane production was determined from six lactating Jersey cows on pasture using both techniques. Methane output data from the LMD had a higher (0.6 *vs*. 0.4) between-cow coefficient of variation compared with data obtained from the SF_6 technique. Methane production as measured by the SF_6 technique (348 g/d) was higher compared with the LMD technique (82.6 g/d). Findings of this study indicate that there is a need to improve the LMD operating protocol and scale-up factors to accurately convert CH_4 concentration (ppmv.m) to CH_4 production (g/d).

In conclusion, this research has provided an understanding of the use and potential of concentrate supplementation as CH_4 mitigation strategy for dairy cows in pasture-based systems, and has shown that dietary nitrate has the potential as CH_4 mitigation strategy for dairy cows in pasture-based systems. Furthermore, this research has provided a novel back-mounted harness for grazing dairy cows in facilitating the SF_6 technique in enteric CH_4 measurement.

OPSOMMING

KWANTIFISERING EN VERMINDERING VAN RUMEN METAANPRODUKSIE DEUR JERSEY-KOEIE OP WEIDING

deur

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Sleutelwoorde: kweekhuisgas-inventaris; kragvoerpeil; dieetnitraat; SF_6 tegniek

Toenemende bewyse van aardverwarming het die behoefte versterk om nasionale kweekhuisgas-inventarisse akkuraat te verifieer en om op plaasvlak kweekhuisgas-verminderingstrategieë te ontwikkel. Landbou is bekend daarvoor dat dit aansienlik bydra tot kweekhuisgasse, met herkouers wat geïdentifiseer word as die belangrikste bron van mensgemaakte metaanvrystellings. Metaan is veral ’n sterk kweekhuisgas. Verskeie metaan-verminderingstrategieë is ontwikkel, maar min het in die verkryging van direkte resultate, doeltreffendheid, volhoubaarheid en praktiese waarde – almal noodsaaklik vir die toepassing daarvan op plaasvlak – geslaag. Kragvoeraanvulling en dieetnitraat-toevoeging is geïdentifiseer as metaan-versagtingstrategieë, wat waarskynlik op plaasvlak toegepas kan word. Alhoewel laasgenoemde metaan-verminderingstrategieë ekstensief op beeste in intensiewe aanhouding geëvalueer is, bestaan daar beperkte navorsing oor die effek daarvan op weiding-gebaseerde melkbeeste, spesifiek oor verskillende seisoene.

Die doel van hierdie studie was om metaan-emissies direk vanaf weiding-gebaseerde Jersey-koeie te meet, terwyl die effek van kragvoeraanvulling en dieetnitraat-toevoeging as metaan-verminderingstrategieë op metaan-emissies, koeiproduksie-prestasie en rumenfermentasie bepaal word. ’n Verdere doel van hierdie studie was om die velskade

wat deur die huidige swael hexafluoriedgas (SF_6)-tegniek-rugharnasse veroorsaak word, te verminder of te elimineer, en metaandata afkomstig van die SF_6-tegniek te vergelyk met metaandata afkomstig van 'n korttermynmetingstegniek.

Die eerste proef ondersoek die uitwerking van kragvoerpeil (0, 4 en 8 kg per koei per dag, soos gevoer) op metaan-emissies, koeiproduksie-prestasie en rumenfermentasie van melkkoeie wat kikoejoe (*Pennisetum clandestinum*)-dominante somerweiding bewei. Sestig Jersey-koeie (waarvan nege gekanuleerde koeie was) is gebruik in ’n ewekansige, volledige blokontwerp met die gekanuleerde koeie in ’n 3 × 3 Latynse-vierkantontwerp. Totale droëmateriaal-inname (DMI), melkopbrengs, energie-gekorrigeerde melk (ECM) en melklaktose-inhoud het toegeneem, terwyl weiding-DMI met toenemende kragvoerpeil afgeneem het. Metaanproduksie (323 tot 378 g/d) het lineêr toegeneem, terwyl metaanopbrengs (g/kg DMI) en metaan-intensiteit (g/kg melkopbrengs en ECM) met toenemende kragvoerpeil afgeneem het. Diurnale ruminale pH, droëmateriaal- (DM) en vesel- (NDF)-verdwyning het afgeneem terwyl bottersuur lineêr en kwadraties met toenemende kragvoerpeil verhoog het. Daar is tot die gevolgtrekking gekom dat ’n hoë kragvoerpeil, enteriese metaanproduksie van koeie wat kikoejoe-dominante weiding wei gedurende die somer met 17% kan verhoog, maar metaanopbrengs met 14% en metaanemissies per eenheid melkopbrengs en ECM met onderskeidelik 39% en 41% kan verminder.

Die tweede proef was in wese ’n herhaling van die eerste proef, maar op meerjarige raaigras (*Lolium perenne*)-dominante weiding, om vas te stel of die metaan-vermindering doeltreffendheid van kragvoeraanvulling beïnvloed is deur seisoenale verandering in weidingsamestelling. Sestig Jersey-koeie (waarvan ses gekanuleerde koeie was) is gebruik. Totale DMI, melkopbrengs, ECM, melklaktose-inhoud en weiding DMI reaksie was soortgelyk aan die vorige proef, maar in hierdie proef het die melkvetinhoud afgeneem met toenemende kragvoerpeil. Vlugtige vetsuurkonsentrasies en ruminale pH is meestal nie deur behandeling beïnvloed nie, terwyl DM verdwyning afgeneem het en NH_3-N konsentrasie met toenemende kragvoerpeil toegeneem het. Metaanproduksie (258 tot 302 g/d) en metaanopbrengs was onveranderd, terwyl metaan-intensiteit lineêr met toenemende kragvoerpeil afgeneem het. Daar is tot die gevolgtrekking gekom dat kragvoeraanvulling die potensiaal het om metaan-emissies per eenheid melkopbrengs van koeie wat in die lente meerjarige raaigras-dominante weiding wei, met 20% effektief te verminder.

Die derde proef ondersoek die effek van dieetnitraat-toevoeging (0, 11 en 23 g nitraat/kg DM; kontrole, lae nitraat en hoë nitraat, onderskeidelik) op metaan-emissies,

koeiproduksie-prestasie en rumenfermentasie van melkkoeie op kikoejoe-dominante weiding wat ongeveer 3 g nitraat/kg DM bevat. Vier-en-vyftig Jersey-koeie is in ’n ewekansige, volledige blokontwerp gebruik, met ses addisionele gekanuleerde koeie wat in ’n 3 × 3 Latynse-vierkantontwerp gebruik is. Kragvoer is teen ’n peil van 5.4 kg DM/koei per dag gevoer en geformuleer om iso-proteïen te wees deur ureum gedeeltelik te vervang met nitraat. Koeie is geleidelik oor ’n drie week periode tot die kragvoer aangepas. Alhoewel die totale DMI onveranderd was, het die hoë nitraatbehandeling DMI en melkopbrengs verminder, maar terselfdertyd weiding DMI verhoog. Verder is rumenfermentasie nie nadelig beïnvloed nie. Daaglikse metaanproduksie (313 tot 280 g/d), metaanopbrengs (21.8 tot 18.7 g/kg DMI) en metaan-energie per bruto energie-inname (Y*m*; 6.9 tot 5.9%) het geneig om lineêr met toenemende dieetnitraat-toevoeging te verminder. Daar is bevind dat dieetnitraat wat aan weidende melkkoeie gevoer is, belowend lyk as metaan-verminderingstrategie.

Die vierde proef was ’n herhaling van die derde proef, maar op meerjarige raaigras-dominante weiding en met slegs twee behandelings wat in wese ’n ander eksperimentele ontwerp tot gevolg het. Die hoë nitraatbehandeling in die derde proef is, weens die waargenome gedeeltelike weiering van kragvoer en verminderde melkopbrengs, nie herhaal nie. Twee-en-dertig intakte en agt rumen-gekanuleerde Jersey-koeie was onderworpe aan ’n gerepliseerde 2 × 2 Latynse-vierkantontwerp. Koeie was aangevul met een van twee kragvoerbehandelings wat ureum (ureumbehandeling) of ureum en nitraat (nitraatbehandeling) bevat het, wat onderskeidelik 0.3 en 15.2 g nitraat/kg DM bevat het. Die weiding het ongeveer 7.3 g nitraat/kg DM bevat. Totale DMI, melkopbrengs, metaanproduksie (400 en 405 g/d) en meeste rumenfermentasie-parameters was nie deur behandeling beïnvloed nie. Dieetnitraat het melk-komponente verhoog (behalwe vir melkproteïeninhoud). Minder effekte op ruminale pH is waargeneem met ’n toenemende neiging tot die nitraatgroep. Daar is tot die gevolgtrekking gekom dat hoewel dieetnitraat-toevoeging nie ’n effektiewe metaan-verminderingstrategie vir weidende melkkoeie is nie, stygings in melkvet- en melklaktose-inhoud verwag kan word.

Die vyfde proef het gefokus op die verbetering van die rugharnas van die SF_6-tegniek gedurende die vier hoofproewe, ten opsigte van die vermindering van velletsels wat deur die harnas opgelê is. Ten slotte is ’n koste-effektiewe rugharnas vir weidende melkkoeie ontwerp wat die SF_6-tegniek fasiliteer in die meting van rumen metaan-emissies en in die proses velletsels vermy.

Die sesde proef was ’n vergelykingstudie tussen metaan-emissiesyfers soos gemeet deur die LMD- en SF_6-tegniek van lakterende melkkoeie wat meerjarige raaigras-dominante weiding bewei. Metaanproduksie van ses lakterende Jersey-koeie op weiding is deur albei tegnieke bepaal. Metaan-uitsetdata van die LMD-tegniek het ’n hoër (0.6 *vs.* 0.4) tussenkoei-koëffisiënt van variasie, in vergelyking met data verkry deur die SF_6-tegniek. Metaanproduksie, soos gemeet deur die SF_6-tegniek (348 g/d), was hoër in vergelyking met die LMD-tegniek (82.6 g/d). Bevindinge van hierdie studie dui daarop dat daar ’n behoefte is om die LMD operasionele protokol en opskaal-vergelyking te verbeter, om metaankonsentrasie (ppmv.m) akkuraat na metaanproduksie (g/d) om te skakel.

Ten slotte het hierdie navorsing ’n beter begrip van die gebruik en potensiaal van kragvoeraanvulling as metaan-verminderingstrategie vir melkkoeie in weiding-gebaseerde stelsels gegee en getoon dat dieetnitraat-toevoeging die potensiaal het as metaan-verminderingstrategie vir melkkoeie, wat subtropiese gewasse soos kikoejoe wei. Verder het hierdie navorsing ’n nuwe rugharnas vir weidende melkkoeie in die fasilitering van die SF_6-tegniek in metaanmeting voorsien.

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LIST OF ABBREVIATIONS

ADF	acid detergent fibre
BCS	body condition score
BW	body weight
CH_4	methane
CP	crude protein
CV	coefficients of variation
DHA	daily herbage allowance
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
ECD	electron-capture detector
ECM	energy-corrected milk
FCM	fat-corrected milk
FID	flame-ionisation detector
FO	faecal output
GE	gross energy
GHG	greenhouse gas
ID	inner diameter
iNDF	indigestible neutral detergent fibre
*IV*OMD	*in vitro* organic matter digestibility
LMD	laser methane detector
ME	metabolisable energy
MUN	milk urea nitrogen
N	nitrogen
NDF k_d	rate of neutral detergent fibre disappearance
NDF	neutral detergent fibre
NFC	non-forage carbohydrate
NIWA	national institute of water and atmosphere
NPN	non-protein nitrogen
NRF	National Research Foundation

OD	outer diameter
OMD	organic matter digestibility
OMI	organic matter intake
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
SCC	somatic cell count
SD	standard deviation
SED	standard error of difference
SEM	standard error of mean
SF_6	sulphur hexafluoride
TiO_2	titanium dioxide
TMR	total mixed ration
Y*m*	methane energy per gross energy intake

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CHAPTER 1

Introduction

1.1 PROBLEM STATEMENT

1.1.1 Context of the problem

The global livestock sector emits approximately 7.1 Gt of carbon-equivalent per annum, with enteric methane (CH_4) from ruminants comprising approximately 39% of the sectors emissions (Gerber et al., 2013), making the livestock sector a major contributor to the build-up of CH_4 emissions in the atmosphere. Adding to that, methane is a damaging greenhouse gas (GHG) with 28 times the global warming potential of carbon dioxide over a given time period of 100 years (Myhre et al., 2013) and represents a loss of energy that could have been converted into animal products. In South Africa, the cattle industry produced 964 Gg of CH_4 emissions during 2010, of which 13.5% was represented by the dairy sector mainly in the form of enteric CH_4 emissions (du Toit et al., 2013). This was obtained by means of tier 2 methodologies as described by the IPCC (2006). In response, some government instances worldwide enforced a carbon tax policy or a carbon credit incentive with the aim to reduce GHG emissions. South Africa is one of the countries that planned for an agricultural carbon tax scheduled for end of 2020 with the aim to reduce their GHG emissions. The need to accurately verify national greenhouse gas (GHG) inventories and validate on-farm GHG mitigation strategies is ever increasing, with global ruminant numbers accumulating on average by 26.9 million on an annual basis since 1961 (FAO, 2016). Several CH_4 mitigation strategies on animal nutrition have been proposed and extensively reviewed (Hristov et al., 2013). However, nutritional CH_4 mitigation strategies applied on pasture-based systems is limited. Since CH_4 produced by dairy cows on pasture-based systems contribute substantially to GHG in the atmosphere, quantifying and mitigating these emissions are essential.

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1.1.3 Research gap

Methane emissions have not yet been directly measured from grazing dairy cows in South Africa. In doing so, the national GHG inventory of South Africa can be improved by updating estimated (tier 2 methodologies) CH_4 emissions from dairy cows with directly measured CH_4 emissions (tier 3 methodologies).

Limited research is available on concentrate supplementation as enteric CH_4 mitigation strategy in pasture-based dairy cows especially on the effect of seasonal changes in pasture composition. More insight is needed on rumen fermentation to better understand the functionality of the latter enteric CH_4 mitigation strategy. The addition of rumen parameters along with CH_4 parameters and production parameters of grazing dairy cows will be novel research. Furthermore, no research has focused on the use of dietary nitrate as CH_4 mitigation strategy in pasture-based dairy cows.

The fundamental elements of the SF_6 technique, such as the permeation tube and sampling line with flow restrictor and gas collection vessel have been well documented. However, the back-mounting options for the gas collection vessel need yet to be standardised in terms of animal welfare.

A comparison study between the SF_6 technique and the laser methane detector (LMD) technique has not yet been done. This is important to identified measurement and accuracy issues and provides opportunity for improvement of the LMD technique.

1.2 RESEARCH AIM, OBJECTIVES AND QUESTIONS

The aim of this study is to directly measure CH_4 emissions from pasture-based Jersey cows grazing pasture, while determining the effect of concentrate feeding level and dietary nitrate addition as CH_4 mitigation strategies on CH_4 emissions, cow performance and rumen fermentation. Additionally, this study aimed to reduce or eliminate animal skin abrasions imposed by current back-mounted harnesses facilitating the SF_6 technique, and to compare CH_4 data derived from the SF_6 technique with that of a short-term measurement technique.

The following objectives are set to reach the aim:

- Individual CH_4 emissions from grazing dairy cows will be directly measured by using the SF_6 technique.
- Methane mitigation trials will be repeated during late-summer and early-spring when the pasture component differs.
- The back-mounted harness of the SF_6 technique will be continuously improved over the series of trials to the point where it functions successfully in terms of animal welfare.
- A comparison study between the SF_6 technique and LMD technique (short-term measurement technique) will be implemented by measuring CH_4 emissions from grazing dairy cows using both techniques.

The aim and objectives of this study intend to address the following research questions:

- Is there a seasonal effect on enteric methane output from grazing Jersey cows?
- Is it possible to mitigate enteric methane from grazing dairy cows without impairing animal production?
- Can concentrate supplementation effectively reduce CH_4 emissions from grazing dairy cows?
- Can dietary nitrate addition effectively reduce CH_4 emissions from grazing dairy cows?
- Can the SF_6 technique be further improved in terms of animal welfare?
- How does CH_4 emissions data derived from the SF_6 technique compare with other CH_4 measuring techniques used in South Africa, such as the laser methane detector technique?

1.3 APPROACH

Four trials were designed to reach the main aim of the study. Two trials each focused on concentrate feeding level and dietary nitrate addition as CH_4 mitigation strategy for cows grazing summer and spring pasture, respectively, to account for seasonal changes in pasture composition; a fifth trial focused on improving the back-mounted harness of the SF_6 technique in terms of animal welfare throughout the four main trials; and a sixth trial compared the LMD technique with the SF_6 technique to measure CH_4 emissions from grazing dairy cows.

1.4 RESEARCH GOALS

The goal of this study is to improve the South African greenhouse gas inventory by providing accurate directly measured enteric CH_4 emissions from grazing dairy cows and to identify a practical CH_4 mitigation strategy that can reduce CH_4 emissions from grazing dairy cows by as much as 15% without impairing animal production and health. The secondary goal of this study is to completely eliminate the occurrence of animal skin

abrasions and lesions caused by conventional back-mounted harnesses facilitating the SF_6 technique, and to develop an improved LMD scale-up equation for CH_4 production.

1.5 RESEARCH CONTRIBUTION

Results obtained from this study can be used to improve the accuracy of the greenhouse gas inventory of the pasture-based South African dairy sector by being able to implement tier 3 methodologies. Furthermore, results from this study will provide insight on concentrate feeding level and dietary nitrate addition as CH_4 mitigation strategies for pasture-based dairy farmers. Results may also promote early adoption of CH_4 mitigation strategies by dairy farmers, which will in return enhance dairy cow production efficiency, optimise resources, lower on-farm carbon footprint and possibly exempt the upcoming South African agricultural carbon tax. Additionally, milk production and intake results from this study can be used for future meta-analysis studies in developing robust prediction equations for pasture intake and milk production per season and may have application to grazing based dairy sectors in other countries.

1.6 LAYOUT OF DISSERTATION

The layout of this dissertation is in accordance with the requirements of the University of Pretoria. The author guidelines of Animal Feed Science and Technology were followed for language, style and reference formatting. This dissertation consists of an introduction chapter followed by six chapters each representing an individual manuscript, a conclusion chapter and an addendum chapter. Literature was reviewed in each manuscript chapter; therefore to keep repetition to a minimum and to avoid an unnecessary long dissertation, a standalone literature review was not included. However, due to the multiple manuscript layout of this dissertation some repetition between chapters is evident. Some manuscript chapters have already been published, while other chapters are still in the process of being reviewed by peer reviewed journals as indicated at the top of each chapter. The addendum chapter represents a standard operating protocol for the custom SF_6 technique implemented throughout this study.

1.7 RESEARCH OUTPUTS

1.7.1 Popular publications (13)

van Wyngaard, J.D.V., 2014. *Metaangasvrystelling van suiwelkoeie – van agter of van voor?*. George Herald, Mossel Bay Advertiser, Southern Cape Forum, Knysna-Plett Herald, Graaff-Reinet Advertiser, Oudtshoorn Courant and www.agrieden.co.za. 8 May.

van Wyngaard, J.D.V., 2014. *Navorsingstendense in die voerbedryf*. Veeplaas Tydskrif 5 (12), 57.

van Wyngaard, J.D.V., 2015. Methane production from cows on pasture. Afgriland 59 (6), 18–19.

van Wyngaard, J.D.V., 2016. *Suiwelkragvoer vir melkkoeie op weiding: Hoeveel is genoeg?*. AgriCulture/Kultuur 36, 63–65.

van Wyngaard, J.D.V., 2016. Dairy concentrate for cows on pasture: what is the limit?. Natal Study Group visit, booklet. Outeniqua Research Farm, George, 13 Jun. pp. 20–21.

van Wyngaard, J.D.V., 2016. Methane production from cows on pasture. Natal Study Group visit, booklet. Outeniqua Research Farm, George, 13 Jun. pp. 20–21.

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van Wyngaard, J.D.V., 2017. Carbon tax on the horizon. The Dairy Mail 24 (3), 82–87.

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van Wyngaard, J.D.V., 2017. Reducing the methane emissions of cattle. Farmer’s Weekly 17027, 26–27.

1.7.2 Formal presentation by invitation (3)

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2016. Is carbon tax a reality for dairy farmers?. Outeniqua Research Farm Information Day: Milk production from planted pastures. Outeniqua Research Farm, George. 19 Oct.

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2016. How to reduce on-farm enteric methane production. Outeniqua Research Farm Information Day: Milk production from planted pastures. Outeniqua Research Farm, George. 19 Oct.

van Wyngaard, J.D.V., 2017. Methane measurement on grazing cows. China Animal Agriculture Association delegate visit to SA. Outeniqua Research Farm, George. 28 Apr.

1.7.3 Radio talk (1)

van Wyngaard, J.D.V., 2014. *Metaangasproduksie van suiwelkoeie op weiding*. Radio Elsenburg, RSG. 4 Apr.

1.7.4 Peer-reviewed scientific journal articles (2 published)

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2018. Technical note: A Simple back-mounted harness for grazing dairy cows to facilitate the sulfur hexafluoride tracer gas technique. J. Dairy Sci. 101: 2655–2658.

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2018. Effect of concentrate level on enteric methane emissions, production performance and rumen fermentation of dairy cows grazing summer pasture. Submitted for review to J. Dairy Sci. on 21 Dec. 2017. Status: accepted.

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2018. Effect of concentrate feeding level on methane emissions, production performance and rumen fermentation of Jersey cows grazing ryegrass pasture during spring. Anim. Feed Sci. Technol. 241, 121–132.

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1.7.5 Peer-reviewed congress contributions (12)

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2015. Evaluation: Sulphur hexa-fluoride tracer gas technique for estimating methane emissions from grazing Jersey cows. Proc. 48th South African Society for Animal Science congress. Umfolozi Hotel, Casino and Conference Resort, Empangeni, KZN. 21–23 Sept.

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2016. Methane research in the dairy sector of South Africa using the ERUCT technique. Proc. 6th Greenhouse Gas and Animal Agriculture Conference (GGAA2016), Pullman Albert Park, Melbourne, Australia. 14–18 Feb.

du Toit, C.J.L., van Niekerk, W.A., Meissner, H.H., van Wyngaard, J.D.V., 2016. Direct greenhouse gas emissions from South African livestock. Proc. 6th Greenhouse Gas and Animal Agriculture Conference (GGAA2016), Pullman Albert Park, Melbourne, Australia. 14–18 Feb.

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van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2016. Predicting methane emissions of Jersey cows on pasture. Proc. 49th South African Society for Animal Science congress, Spier Hotel and Congress Centre, Stellenbosch, RSA. 6–7 Jul.

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2017. Dairy concentrate feeding level – the sweet spot. Proc. 10th South African Large Herds Conference, Champagne Sports Resort, Drakensberg, RSA. 5–7 Jun.

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van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2017. Measured *vs.* predicted enteric methane emissions of pasture-based dairy cows. Proc. 50th South African Society for Animal Science congress, Boardwalk Conference Centre, PE, RSA. 18–21 Sep.

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CHAPTER 2

Effect of concentrate level on enteric methane emissions, production performance and rumen fermentation of Jersey cows grazing kikuyu-dominant pasture during summer

2.1 ABSTRACT

The effect of concentrate feeding level on enteric methane (CH_4) emissions from cows grazing medium quality summer pasture is yet to be investigated. Sixty multiparous Jersey cows (nine rumen-cannulated) were used in a randomised complete block design study (with the cannulated cows in a 3 × 3 Latin square design) to investigate the effect of concentrate feeding level (0, 4, and 8 kg/cow per day; as fed basis) on enteric CH_4 emissions, production performance and rumen fermentation of dairy cows grazing summer pasture (17 cows plus three cannulated cows per treatment). Enteric CH_4 emissions were measured from 11 cows per treatment group during one 7-d measurement period using the sulphur hexafluoride tracer gas technique. Pasture dry matter intake (DMI) was determined parallel with the CH_4 measurement period using TiO_2 as external marker, while milk yield, milk composition, cow condition, and pasture pre- and post-grazing measurements were also recorded. Daily total DMI (11.2 to 15.6 kg/cow), milk yield (9.1 to 18.2 kg/cow), energy-corrected milk (ECM; 11.2 to 21.6 kg/cow), and milk lactose content (44.1 to 46.7 g/kg) increased linearly, while pasture DMI (11.2 to 8.4 kg/cow) decreased linearly with increasing concentrate feeding level. Daily CH_4 production (323 to 378 g/d) increased linearly due to the increase in total DMI, while CH_4 yield (29.1 to 25.1 g/kg of DMI) and CH_4 intensity (35.5 to 21.1 g/kg of milk yield; and 28.8 to 17.6 g/kg of ECM) decreased linearly with increasing concentrate feeding level. Diurnal ruminal pH (6.45 to 6.32), and *in sacco* DM and neutral detergent fibre disappearance decreased linearly. Acetic and propionic acid were unaffected by treatment, while butyric acid (5.21 to 6.14 m*M* %) increased linearly and quadratically with increasing concentrate feeding level. It was concluded that a high concentrate feeding level not only increases animal efficiency but is

moreover a viable CH_4 mitigation option for dairy cows grazing kikuyu-dominant pasture in late-summer when pasture is inherently fibrous.

Key words: CH_4 measurement; kikuyu; methane mitigation; pasture-based; SF_6

2.2 INTRODUCTION

Climate change transforms and threatens current and future global natural resources. Globally, the livestock sector is responsible for approximately 14.5% of all anthropogenic greenhouse gas (GHG) emissions of which approximately 39% is in the form of CH_4 (Gerber et al., 2013). In addition, CH_4 is a potent GHG with 28 times the greenhouse potential of carbon dioxide (CO_2) over a 100 year period (Myhre et al., 2013). In South Africa, the cattle industry produced approximately 27.1 megatonnes of CO_2-equivalent during 2010 of which 98.6% was represented by enteric CH_4 emissions (du Toit et al., 2013). The need to verify national GHG inventories and to validate on-farm GHG mitigation strategies has become a growing concern on an international level.

It is well documented that concentrate in diets has a profound negative effect on CH_4 intensity (g/kg of milk or meat) when fed at increasing levels (Beauchemin et al., 2008; Martin et al., 2010; Knapp et al., 2014). The decrease in CH_4 intensity can mainly be ascribed to a shift in the NDF:NFC ratio that, in combination, provides for a higher net energy intake that can favour animal production, and alters rumen fermentation in such a way that alters CH_4 production. Most grazing studies that evaluated concentrate supplementation as CH_4 mitigation strategy in dairy systems utilised high quality pasture, predominantly ryegrass pasture or a dominant mix thereof during spring (Jiao et al., 2014; Muñoz et al., 2015). None of these grazing studies compared CH_4 emissions from cows fed concentrate to that of cows on a pasture-only diet; also, none explored the treatment effect on rumen function. Including a pasture-only treatment is not only important for control comparison purposes but also for GHG inventory resolutions because there are dairy farmers that still implement once-a-day milking on pasture without concentrate supplementation. Although it is common practice to graze year-round in the southern hemisphere and that the challenge of seasonal variation in pasture availability and nutritive quality is well documented (Roche et al., 2009), little is known on the effect of summer

pasture (low to medium quality), with or without supplementation, on CH_4 emissions of dairy cows.

Thus, the aim of the study was to determine the effect of different concentrate levels (including a zero level) on CH_4 emissions, production performance and rumen fermentation of Jersey cows grazing kikuyu-dominant pasture during summer. We hypothesised that dairy cows supplemented with concentrate grazing medium quality pasture in summer will emit less CH_4 emissions, yield and intensity compared with cows receiving pasture only.

2.3 MATERIALS AND METHODS

2.3.1 Location and Animal Ethical Clearance

The study was conducted at the Outeniqua Research Farm of the Western Cape Department of Agriculture, Western Cape, George (33°58´S, 22°25´E) during the beginning of 2017. The area is characterised by a coastal temperate climate with long-term mean annual precipitation of 732 mm, spread throughout the year, and a mean daily minimum and maximum temperature range of 7°C to 15°C, and 18°C to 25°C, respectively. The soil was characteristic of a Podzol soil type as described in detail by Swanepoel et al. (2013). Institutional animal care and use was obtained from the Western Cape Department of Agriculture (Elsenburg, South Africa) before commencement of the study (DECRA approval number: R114/115) and unnecessary discomfort to the animals was avoided at all times.

2.3.2 Animals, Experimental Design and Treatments

Fifty-one multiparous (mean parity, 4.1 ± 1.57 SD) Jersey cows were selected from the Outeniqua dairy herd with a mean pre-experimental milk yield of 16.5 (±1.97 SD) kg/d and a mean of 99 (±46) DIM at the onset of the study. Cows were blocked (17 blocks) according to pre-experimental milk yield, DIM, and parity (covariate data), in one of three treatment groups on January 25, 2017. Additionally, nine lactating rumen-cannulated

Jersey cows (previously fitted with Bar Diamond #1C rumen cannulae; Bar Diamond Inc, Parma, Idaho, USA) were randomly, evenly allocated to the same three groups. Each treatment group (consisting of 20 cows) was then randomly assigned to one of three treatments. Treatments consisted of three concentrate feeding levels: 0, 4 and 8 kg/cow per day (as fed basis). Concentrate was offered individually to cows in pellet form in two equal portions during milking. The ingredient composition of the concentrate offered was (g/kg as fed basis) as follows: 740 maize, 175 soybean oilcake, 50 sugarcane molasses, 22 limestone ($CaCO_3$), 3 monocalcium phosphate, 6 salt, 3 magnesium oxide and 1 trace minerals and vitamins (containing 4 mg of Cu/kg, 10 mg of Mn/kg, 20 mg of Zn/kg, 0.34 mg of I/kg, 0.2 mg of Co/kg, 0.06 mg of Se/kg, 6×10^6 IU of vitamin A/kg, 1×10^6 IU of vitamin D3/kg, and 8×10^3 IU of vitamin E/kg). Cows were subject to a 14 d dietary adaption period that started February 9, followed by a 47 d data collection period that commenced February 23 and ended April 11. The rumen-cannulated cows formed part of a 3 × 3 Latin square design with 20 d periods (14 d adaptation and 6 d data collection).

2.3.3 Pasture and Grazing Management

Cows grazed kikuyu-dominant (*Pennisetum clandestinum*) pasture as one group throughout the study period during late summer. The experimental paddock size was 8.55 ha with permanent sprinkler-irrigation divided into 15 m × 150 m strips with electric fence. Pre-experimental botanical composition of the pasture (at point of grazing; n = 18) comprised 49.8% kikuyu, 32.4% perennial ryegrass (*Lolium perenne*), 14.0% other grass (*Lolium multiflorum* and *Paspalum dilatatum*), 2.1% white clover (*Trifolium repens*), and 1.7% broad-leaf weeds. Irrigation was scheduled by irrometer tensiometers (Calafrica SA, Nelspruit, South Africa) installed at a depth of 150 mm. Irrigation was initiated at a tensiometer reading of –25 kPa and ended at a reading of –10 kPa. Pasture strips were top-dressed with limestone ammonium nitrate (containing 280 g of N/kg) after each grazing at a rate of 42 kg of N/ha. Cows were grazed in a rotational system with fresh pasture allocated twice daily after milking, with grazing areas being back-fenced. The daily herbage allowance (DHA) was continuously adjusted throughout the study to ensure a target post-grazing height of 5.5 cm aboveground. This was achieved by measuring pre-

and post-grazing sward height with a rising plate meter (Jenquip folding plate pasture meter; Jenquip, Feilding, NZ) by taking 100 readings in a zigzag pattern across the grazing area. Mean aboveground pre- and post-grazing pasture yield were estimated using the following site-and-season-specific linear regression equation: Pasture yield (kg of dry matter (DM)/ha) = [87.8 × sward height (rising plate meter reading)] – 32.7 (R^2 = 0.94). According to this equation, a residual pasture yield of 933 kg of DM/ha was attained at the target post-grazing height of 5.5 cm aboveground.

2.3.4 Measurements

2.3.4.1 Animal performance

All cows were milked twice daily at 0700 h and 1500 h in a dairy parlour equipped with a 20-point swing over milking machine with weigh-all electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition was determined weekly from composite morning and afternoon milk samples using a Milkoscan FT+ milk analyser (FOSS Analytical, DK-3400 Hillerød, Denmark) for determining milk fat, milk protein, milk lactose and milk urea nitrogen (MUN), and a Fossomatic FC (FOSS Analytical) for determining somatic cell count (SCC). Fat corrected milk (FCM) standardised to 4.0% fat was calculated using the equation of Gaines (1928): FCM = [0.4 × milk yield (kg)] + [15 × milk fat (kg)] and ECM was calculated using the equations of Tyrrell and Reid (1965) as presented by Muñoz et al. (2015). Milk parameters of the rumen-cannulated cows were excluded from the treatment group mean due to the cross-over design.

All cows were weighed over two consecutive days and body condition score (BCS) recorded before afternoon milking at the start and the end of the study period. Bodyweight (BW) was recorded electronically with a fixed weighing scale (Tru-Test EziWeigh v. 1.0 scale, 0.5 kg accuracy, Auckland, New Zealand) and BCS was determined using the 1 to 5 scale scoring system of Wildman et al. (1982).

2.3.4.2 Dry matter intake

Individual pasture DMI of intact cows was estimated with the use of titanium dioxide (TiO_2) as an external marker to determine faecal output (FO) and indigestible NDF (iNDF)

as an internal marker to determine forage digestibility. Cows in block 1 to 11 of each treatment group were used for estimating pasture DMI and one additional cow per treatment was included for background TiO_2 analysis. The TiO_2 method (dosing 3 g of TiO_2/cow twice daily for 10 d and collecting a.m. and p.m. faecal samples on d 5 to d 10) of Pinares-Patiño et al. (2008) was implemented from March 22 to 31, 2017. Faecal samples were immediately oven dried (65°C, 72 h), pooled within-animal and analysed for TiO_2 concentration by the method of Myers et al. (2004). Faecal output was calculated from the daily TiO_2 dose and TiO_2 concentration in faeces as described by de Souza et al. (2015).

During the DMI measurement period, representative pasture samples were cut daily before p.m. milking on the successive grazing-strip, at a stubble height of approximately 3 cm aboveground. Samples were immediately oven dried (55°C, 72 h), pooled and milled to pass a 1 mm sieve. The iNDF concentration of the concentrate, forage and faecal samples was determined according to Krizsan et al. (2015) by incubating samples *in situ* for 288 h in polyester bags (07-11/5 Sefar Petex cloth, Sefar AG, Heiden, Switserland) with a sample size to surface area ratio of 12 mg/cm^2. After incubation, bags were inserted in an Ankom^{200} fibre analyser (Ankom Technology Corp., Fairport, NY) with the inclusion of heat-stable α-amylase and anhydrous sodium sulfite following the procedure of Robertson and van Soest (1981). Finally, pasture DMI was calculated using the following equation (Cabral et al., 2014): Pasture DMI (kg/d) = [[FO (kg/d) × iNDF faeces (kg/kg)] – iNDF concentrate intake (kg/d)]/iNDF forage (kg/kg).

2.3.4.3 Enteric methane emissions

Cows from block 1 to 11 (highest milk producers) of the experimental cow group were selected for enteric CH_4 measurement. Methane emissions from individual cows were recorded parallel to the DMI measurement period using the SF_6 technique as described by O'Neill et al. (2011) for free-ranging dairy cattle. This measurement period prolonged for a maximum of seven consecutive days (March 26 to April 2) to ensure at least four representative 24 h gas samples per cow after the completion of the measurement period. Empty permeation tubes (P&T Precision Engineering Ltd., Unit 2, Naas Industrial Estate, Naas, Co. Kildare, W91 KA4C, Ireland) used within this study were filled with 3.0 (±0.19

SD) g of SF_6 gas during January 2017. Filled tubes were calibrated in a dry incubator (Labcon Incubator Model FS1M8, The Palms Office Park, Block D, Ground Floor, 391 Main Ave, Ferndale, Johannesburg) set at 39.0°C for 4 wk weighing the tubes (Sartorius BP210S, Sartorius AG, Göttingen, Germany; 0.0001 g accuracy) every third morning to produce a 9-point regression curve ($R^2 > 0.9993$). The experimental mean release rate of the SF_6 gas from the permeation tubes was 4.9 (±0.26 SD) mg/d and ranged from 4.4 to 5.3 mg/d one week prior dosing. The permeation tubes were blocked by release rate and randomly allocated to both experimental treatment and cow within treatment. Tubes were individually placed in a size 10 gelatine capsule (Torpac Inc., 333 Route 46, Fairfield, NJ 07004, USA) and dosed *per os* using a plastic capsule-dose-applicator on March 17 (9 d prior to the measurement period).

Cylindrical, back-mounted polyvinyl chloride (PVC) gas-collection canisters of 1700 mL with an initial sampling rate of approximately 0.54 mL/min were used to continuously sample eructated gasses over a 24 h period. The given sampling rate allowed for the evacuated canister to fill to 45% over a 24 h sampling period. Canisters were mounted on the back of the cows using the back-mounted harness of van Wyngaard et al. (2018a). Canisters were flushed prior use, which encompassed five cycles of evacuating to 98 kPa vacuum, filling with ultra-high purity nitrogen gas (999.99 g of nitrogen/kg) and evacuating again to 98 kPa vacuum. Initial sampling rate was obtained by restricting flow with a stainless-steel capillary tube (1/16" OD x 0.2" ID; YY-RES-21503; LECO Co., Saint Joseph, MI 49085, USA) cut to 50 mm length and crimped using a table top vice-grip until the specified flow was attained.

Background (ambient) concentrations of SF_6 and CH_4 were sampled by using three additional cows without permutation tubes (block 12), equipped with the same saddle and canister as those used by the experimental cows for one exclusion that the flow inlet was on the back of the animal and not above the nostrils. The background cows and the experimental cows were kept in one group at all times (grazing and milking). Background emissions were averaged per day to give a single estimate for all experimental cows. The same oil vacuum gauge (SA Gauge (Pty.) Ltd., Durban, South Africa) was used to measure vacuum of evacuated canisters prior daily connection and removal of sample canisters.

Undiluted gas samples were extracted and subsampled into three 12 mL glass vials (Labco Exetainer, Labco Ltd., Lampeter, Ceredigion, SA48 7HH, UK) from the sample canisters by means of a piston sub-sampler (National Institute of Water and Atmosphere (NIWA) Ltd., Viaduct Harbour, Auckland Central, 1010, NZ) and shipped to NIWA for gas analysis (analysed approximately 14 d after sampling).

Gas samples were analysed using an automated gas analyser equipped with a Gilson Sample Changer (Gilson, Inc., Middleton, WI 53562-0027, USA) modified at NIWA to analyse pressurised air samples in Labco Exetainers, and a GC equipped with a flame-ionization detector (FID) and an electron-capture detector (ECD; Hewlett Packard Model 6890, Palo Alto, CA, USA). Separation of CH_4 and SF_6 from the other air components was achieved using two Alltech Porapak-Q 80-100 mesh columns (3.6 m × 3 mm stainless steel; Grace Davison Discovery Sciences, Deerfield, IL, USA) in parallel configuration, one for each detector. The ECD operated at 400°C and the FID at 250°C using 10% Ar/CH_4 and ultra-high purity nitrogen gas as carrier gas at 30 mL/min flow, respectively. The sample loops were flushed in a direction away from the FID so the CH_4 in the ECD carrier gas was not carried through to the FID. A suite of three standards of SF_6 and CH_4 mixtures (NIWA) were associated with the analyses of each batch. Each of the three standards was run in triplicate prior to each batch's analysis and again at the end of the batch to characterize GC performance. Methane production (g/d) was calculated using equation 2 from the study of Williams et al. (2011).

2.3.4.4 Rumen fermentation

Ruminal pH, fermentation end-products, and *in situ* pasture DM and NDF disappearance were determined using the nine cannulated cows during each 20 d sampling period. Diurnal pH patterns were measured over a 72 h period (10 min frequency) with Indwelling TruTrack pH Data Loggers (Model pH-HR mark 4, Intech Instruments Ltd., Riccarton, Christchurch 8011, NZ) attached to the rumen cannula. The loggers were calibrated with buffer solutions of pH 4 and 9, and verified with pH 7 buffer solution. After calibration, the loggers were placed in distilled water for 18 h where pH was monitored with a calibrated handheld pH logger (pH340i pH meter/data logger attached with a Sentix 41 pH electrode; WTW, 82362 Weilheim, Germany). Any drift in pH recorded during this

time was used to correct the pH data after incubation. A total of 100 mL ruminal fluid was collected from each cow in 8 h intervals (0600, 1400 and 2200 h) using a vacuum pump and a sampling tube placed into the ventral sac of the rumen via the cannula. After sampling, ruminal pH was immediately measured with the handheld pH logger (spot sample pH). Subsequently, samples were filtered through four layers of cheesecloth, subsampled in airtight containers and immediately frozen for subsequent NH_3-N (Broderick and Kang, 1980) and VFA (Filípek and Dvořák, 2009) analysis. Ruminal disappearance of the kikuyu-dominant pasture was determined using the nylon bag procedure of Cruywagen (2006). The bags were incubated for 6, 18 and 30 h to determine DM and NDF disappearance. The rate of NDF disappearance (NDF k_d) was calculated according to van Amburgh et al. (2003). Bag residues were analysed for DM content (AOAC, 2000; method 934.01), NDF content (as described before), and ADL content (Goering and van Soest, 1970).

2.3.5 Feed Sampling and Analysis

Concentrate and pasture (representative of that grazed) samples were collected weekly. A pasture sample consisted of 6 pooled pasture samples cut approximately 3 cm aboveground from the successive grazing-strip. Samples were thoroughly homogenised, dried at 55°C for 72 h (initial DM), ground to pass through a 1 mm sieve (SMC hammer mill) and stored at −18°C pending analyses. Samples were analysed for DM, ash, crude protein (CP; nitrogen (N) content determined using a LECO Trumac^{TM} N Determinator, LECO Corporation, Saint Joseph, MI, USA) and ether extract, according to procedures of AOAC (2000; methods 934.01, 942.05, 968.06 and 920.39, respectively). The NDF content was determined as described before. Acid detergent fibre (using the Ankom^{200} fibre analyser) and acid detergent lignin content were determined according to Goering and van Soest (1970). Samples were also analysed for gross energy (GE; MC–1000 modular calorimeter, Energy Instrumentation, Sandton, South Africa; operator's manual) and *in vitro* organic matter digestibility (*IV*OMD; Tilley and Terry, 1963; using rumen fluid from a rumen-cannulated SA Mutton Merino ram fed good-quality lucern hay). Metabolisable energy (ME) was calculated using the equations of MAFF (1984): $ME_{concentrate} = 0.84 \times GE$

× *IV*OMD and $ME_{pasture}$ = 0.81 × GE × *IV*OMD. Mineral composition was determined according to the procedure of AgriLASA (1998; method 6.1.1).

2.3.6 Statistical Analysis

Milk parameters (yield and composition) and body condition parameters over the course of the study (17 blocks) and for the duration of the CH_4 emissions measurement period (11 blocks) along with DMI and CH_4 emissions parameters were analysed as a randomised complete block design with ANOVA to test for differences between treatment effects. The residuals were acceptably normal with homogeneous treatment variances, except for SCC, which were then log (base 10) transformed. Pre-experimental milk yield (for two months), DIM and parity were used as covariate to test for significant (linear) relationships between the before and after measurements over the course of the study and subsequent for differences between treatment effects. If the relationship was not significant, then ANOVA was used to test for differences between treatment effects on the after measurements. Covariate analysis was done on milk yield, milk fat content, milk protein:fat ratio, milk lactose content and milk lactose yield.

The pH measurements over 24 h, time spent below ruminal pH, *in situ* disappearance and rumen fermentation end-products were analysed as a triplicated 3 × 3 Latin square testing for differences between treatment effects. Time spent below ruminal pH of 6.6, 6.4, 6.2, 6.0, and 5.8 was Poisson distributed and thus analysed with generalised linear model analysis to test for differences between treatment effects.

The recorded daily CH_4 emission of individual cows was averaged to yield a single daily value for each cow representative of the entire sampling period. A 90% successful collection rate was achieved from the 213 gas samples collected. The remainder was lost due to blockages in the capillary flow restrictor, leaking gauges and broken sampling lines during the 24 h collection periods. The modified Z-score was used to identify outlying CH_4 data using norm permeation tube rate (net SF_6 (ppt) divided by the SF_6 release rate of the permeation tube) and CH_4/SF_6 ratio. Data associated with ‘modified Z-scores’ of >3.5 (absolute value) were labelled as outliers. Only 8 outliers were identified with the norm permeation rate.

Treatment means were compared using Tukey's least significant difference test at the 5% level of significance (Snedecor and Cochran, 1980). Data were analysed using the statistical program GenStat (Payne et al., 2014).

2.4 RESULTS

2.4.1 Feed Chemical Composition and Pasture Measurements

The chemical composition of the dairy concentrate and pasture offered averaged across the 7-wk study period and the CH_4 measurement period are presented in Table 2-1. Pasture grazed during the CH_4 measurement period was fairly representative to the pasture grazed throughout the study period, except for having a lower EE, OM digestibility, GE, ME, Ca, P and Na content, and a higher K content.

The pre- and post-grazing measurements of the offered pasture between the 7-wk study period and the CH_4 measurement period were relatively comparable (Table 2-2). Cows were offered pasture at 11.7 kg of DM/cow per day above 3 cm ground level, given a pasture yield of 2.2 t of DM/ha. According to the pre- and post-grazing measurements, cows consumed daily only approximately 52% of the pasture offered.

2.4.2 Milk production, Milk composition and Cow Condition

Milk production and cow condition measurements over the 7-wk study period are presented in Table 2-3. Milk yield, including FCM and ECM, increased linearly and quadratically ($P<0.05$) with increasing level of dairy concentrate. Similarly, milk fat, protein and lactose yield also increased ($P<0.001$) with increasing level of dairy concentrate. A milk response of 1.24 and 0.93 kg of milk/kg of concentrate was achieved between the 0 and 4 kg group, and between the 4 and 8 kg group, respectively. Milk fat content decreased linearly ($P=0.006$) with increasing concentrate feeding level while the pasture-only group produced a greater (+6.1 g/kg; $P=0.016$) milk fat content than the 8 kg group but similar ($P>0.05$) to the 4 kg group.

Table 2-1 Chemical composition of the concentrate offered and of the pasture offered averaged over both the 7-wk study period and methane (CH_4) measurement period (mean ± SD).

Item[1]	Concentrate (n = 6)	Pasture[2]	
		7-wk study (n = 6)	CH_4 measurement period (n = 5)
Initial DM (%)	89.9 ± 0.48	17.8 ± 2.42	17.3 ± 1.27
DM composition (g/kg of DM)			
CP	133 ± 3.09	208 ± 29.8	193 ± 27.3
EE	36.7 ± 4.90	30.1 ± 4.63	26.0 ± 3.65
NDF	107 ± 4.8	574 ± 27.1	591 ± 29.3
ADF	39.4 ± 3.98	293 ± 22.7	299 ± 17.4
Ash	78.3 ± 2.26	104 ± 4.0	114 ± 13.4
*IV*OMD (g/kg of DM)	946 ± 22.2	740 ± 55.4	627 ± 16.6
GE (MJ/kg of DM)	17.2 ± 0.05	18.0 ± 0.35	17.7 ± 0.16
ME (MJ/kg of DM)	13.7 ± 0.34	10.8 ± 0.79	8.98 ± 0.201
Mineral composition			
Ca (g/kg of DM)	15.6 ± 1.16	4.61 ± 0.522	3.85 ± 0.217
P (g/kg of DM)	5.95 ± 0.202	4.20 ± 0.877	3.42 ± 0.581
Mg (g/kg of DM)	4.40 ± 0.215	4.97 ± 0.356	5.07 ± 0.266
K (g/kg of DM)	9.70 ± 0.594	26.1 ± 11.82	42.9 ± 9.19
Na (g/kg of DM)	2.27 ± 0.135	8.25 ± 4.175	1.86 ± 0.256
Mn (mg/kg of DM)	82.6 ± 5.21	34.7 ± 7.00	49.8 ± 4.33
Cu (mg/kg of DM)	27.7 ± 5.34	7.85 ± 2.277	7.58 ± 1.971
Fe (mg/kg of DM)	110 ± 12.0	95.7 ± 27.91	98.7 ± 34.49
Zn (mg/kg of DM)	130 ± 4.7	54.9 ± 7.84	57.2 ± 12.64

[1] DM–dry matter; CP–crude protein; EE–ether extract; NDF–neutral detergent fibre; ADF–acid detergent fibre; *IV*OMD–*in vitro* organic matter digestibility; GE–gross energy; ME–metabolisable energy.
[2] Pasture–kikuyu (*Pennisetum clandestinum*) dominant.

Milk lactose content increased linearly and quadratically (P<0.05) while cows on both the 4 and 8 kg concentrate level had a greater (P<0.001) milk lactose content compared with cows on the 0 kg concentrate level. Milk protein content and SCC were unaffected by treatment. Milk solids content was unaffected by treatment but tended to decrease linearly with increasing concentrate feeding level. Milk protein to fat ratio increased linearly with increasing concentrate feeding level while the 8 kg group had a greater (+0.07; P=0.004) ratio than both the 0 and 4 kg group that were similar. Individual MUN concentrations decreased linearly and quadratically (P<0.001) with increasing concentrate feeding level while both the 0 and 4 kg group produced greater (P<0.001) MUN concentrations than the 8 kg group.

Initial BW and BCS of all the groups were similar. In contrast, BW change and BCS change increased linearly (P<0.05) with increasing concentrate feeding level. The 8 kg group gained more (+17.5 kg of BW/cow; P<0.004) BW than the 0 kg group, while the 4 kg group remained unaffected.

Table 2-2 Pre- and post-grazing measurements of the experimental kikuyu-dominant pasture averaged (±SD) across the 7-wk study period and the methane (CH_4) measurement period.

Item[1]	7-wk study (n = 89)	CH_4 measurement period (n = 13)
Pasture height (cm)		
Pre-grazing	12.7 ± 3.72	11.8 ± 1.46
Post-grazing	5.95 ± 0.98	6.35 ± 0.66
Pasture yield (kg of DM/ha)[2]		
Pre-grazing	2197 ± 653.1	2027 ± 256.4
Post-grazing	1008 ± 172.0	1082 ± 115.1
DHA (kg of DM/d)	11.7 ± 1.49	11.3 ± 0.88
Daily grazed area (m^2/cow)	56.7 ± 13.94	56.3 ± 8.15
Pasture removed (kg of DM/d)	6.11 ± 1.646	5.22 ± 0.917

[1] DM – dry matter; DHA – daily herbage allowance.
[2] Pasture yield kg of DM/ha) = (88 × rising plate meter reading) – 33 (R^2 = 0.94); estimated 3 cm aboveground level using a rising plate meter.

2.4.3 Dry Matter Intake and Enteric Methane Emissions

The effect of concentrate level on DMI and CH_4 emissions, along with the milk production and milk composition recorded during this measurement period, are presented in Table 2-4. Fecal output was unaffected by treatment but tended to decrease linearly with increasing concentrate feeding level. Pasture DMI decreased linearly (P=0.010) with increasing concentrate feeding level where the 0 kg group had a greater (+2.84 kg of DM; P=0.028) pasture DMI than the 8 kg group but similar to the 4 kg group. Conversely, total DMI, DMI as % BW, GE intake and ME intake increased linearly (P<0.05) with increasing concentrate feeding level while NDF intake as % of BW tended to increase linearly with increasing concentrate feeding level. The 8 kg group had a greater (P=0.001; P<0.001; and P=0.002) total DMI, DMI as % of BW and GE intake, respectively, than both the 0 and 4 kg groups that were similar. Feed efficiency (kg of ECM/kg of DMI) increased linearly

(P<0.001) with increasing concentrate feeding level. The 4 and 8 kg group had similar feed efficiencies while being the lowest (P<0.001) for the 0 kg group.

Table 2-3 The effect of concentrate supplementation level on milk production and cow condition of early lactation Jersey cows grazing kikuyu-dominant pasture in late summer during the 7-wk study period (n = 17).

Item[1]	Concentrate level (kg/d as fed)			SEM[2]	P-value[3]		
	0	4	8		Con	Lin	Quad
Milk yield (kg/d)	9.03^{c}	14.0^{b}	17.7^{a}	0.239	<0.001	<0.001	0.035
FCM yield (kg/d)	11.4^{c}	17.4^{b}	20.8^{a}	0.321	<0.001	<0.001	0.003
ECM yield (kg/d)	11.2^{c}	17.1^{b}	20.7^{a}	0.289	<0.001	<0.001	0.002
Milk fat (g/kg)	58.3^{a}	56.6^{ab}	52.2^{b}	1.37	0.010	0.006	0.43
Milk protein (g/kg)	38.0	37.6	37.3	0.51	0.60	0.56	0.42
Milk protein to fat ratio	0.65^{b}	0.66^{b}	0.73^{a}	0.015	0.002	0.002	0.13
Milk lactose (g/kg)	44.6^{b}	46.3^{a}	46.5^{a}	0.26	<0.001	<0.001	0.036
Milk solids (g/kg)	141	140	136	1.65	0.12	0.056	0.42
MUN (mg/dL)	15.5^{a}	16.2^{a}	13.6^{b}	0.357	<0.001	<0.001	<0.001
Log_{10} SCC	2.12	2.01	2.12	0.068	0.41	0.99	0.18
Milk fat yield (kg/d)	0.52^{c}	0.79^{b}	0.91^{a}	0.019	<0.001	<0.001	0.005
Milk protein yield (kg/d)	0.34^{c}	0.52^{b}	0.66^{a}	0.009	<0.001	<0.001	0.17
Milk lactose yield (kg/d)	0.40^{c}	0.65^{b}	0.82^{a}	0.013	<0.001	<0.001	0.035
BW (kg)	385	389	388	6.4	0.91	0.78	0.75
BCS (scale 1 to 5)	2.02	2.03	2.02	0.017	0.79	1.00	0.49
BW change (kg)	-0.82^{b}	4.53^{ab}	16.7^{a}	3.51	0.004	0.001	0.43
BCS change	-0.05^{c}	0.18^{b}	0.29^{a}	0.032	<0.001	<0.001	0.19

^{a,b,c} Row means with different superscripts differ significantly at P<0.05.
[1] FCM–fat-corrected milk; ECM–energy-corrected milk; milk solids = milk fat + milk protein + milk lactose; MUN–milk urea nitrogen; SCC–somatic cell count; BW–body weight; BCS–body condition score.
[2] SEM – standard error of mean.
[3] Con–contrast; Lin–linear; Quad–quadratic.

Daily CH_4 production (g/d) and CH_4 energy were unaffected by treatment but increased linearly (P=0.045) with increasing concentrate feeding level. Individual CH_4 yield (g/kg of DMI), CH_4 intensity (g/kg of milk yield, and ECM) and Y*m* decreased linearly (P<0.05) with increasing concentrate feeding level. Furthermore, CH_4 intensity (g/kg of milk yield) also tended to decrease quadratically with increasing concentrate feeding level. The 8 kg group emitted less (–4 g/kg; P=0.025) CH_4 per kg of DMI than the 0 kg group but similar to the 4 kg group. Methane per kg of milk yield was similar for the

4 and 8 kg groups but greater (P<0.001) for the 0 kg group, whereas CH_4 per kg of ECM decreased (P<0.001) stepwise with increasing concentrate feeding level.

Table 2-4 The effect of concentrate supplementation level on dry matter intake and methane (CH_4) emissions of early lactation Jersey cows grazing kikuyu-dominant pasture in late summer (n = 11).

Item[1]	Concentrate level (kg/d as fed)			SEM[2]	P-value[3]		
	0	4	8		Con	Lin	Quad
BW (kg)	391	389	396	7.5	0.79	0.64	0.63
FO (kg of DM/d)	3.36	2.82	2.78	0.228	0.16	0.087	0.38
Intake							
Pasture DMI (kg/d)	11.2^{a}	9.19^{ab}	8.36^{b}	0.704	0.028	0.010	0.50
Total DMI (kg/d)	11.2^{b}	12.8^{b}	15.6^{a}	0.70	0.001	<0.001	0.50
NDF intake as % of BW	1.69	1.50	1.45	0.095	0.18	0.083	0.54
DMI as % of BW	2.87^{b}	3.30^{b}	3.95^{a}	0.164	<0.001	<0.001	0.58
GE intake (MJ/d)	202^{b}	228^{b}	275^{a}	12.7	0.002	<0.001	0.50
ME intake (MJ/d)	121^{c}	149^{b}	189^{a}	7.6	<0.001	<0.001	0.50
Feed efficiency (kg ECM/kg DMI)	1.01^{b}	1.37^{a}	1.46^{a}	0.065	<0.001	<0.001	0.11
CH_4 emissions							
CH_4 production (g/d)	323	367	378	18.3	0.11	0.045	0.48
CH_4/DMI (g/kg)	29.1^{a}	28.9^{ab}	25.1^{b}	1.09	0.025	0.016	0.18
CH_4/milk yield (g/kg)	35.5^{a}	25.1^{b}	21.1^{b}	1.26	<0.001	<0.001	0.051
CH_4/ECM (g/kg)	28.8^{a}	21.2^{b}	17.6^{c}	0.98	<0.001	<0.001	0.12
CH_4 energy (MJ/d)	17.8	20.3	20.9	1.01	0.104	0.045	0.48
Y*m* (%)	8.91	8.97	7.85	0.341	0.052	0.039	0.17

^{a,b,c} Row means with different superscripts differ significantly at P<0.05.
[1] BW–body weight; FO–faecal output; DM–dry matter; DMI–dry matter intake; NDF–neutral detergent fibre; GE–gross energy; ME–metabolisable energy; ECM–energy-corrected milk; CH_4–methane; CH_4 energy = (55.22 MJ*CH_4 g/d)/1000; Y*m*–methane energy per gross energy intake.
[2] SEM–standard error of mean.
[3] Con–contrast; Lin–linear; Quad–quadratic.

2.4.4 Rumen Fermentation

The effect of concentrate level on diurnal patterns of ruminal pH is presented in Figure 2-1. It was evident that ruminal pH decreased (P<0.05) for cows in the 8 kg group 1 h after a.m. feeding of concentrate and remained lower (P<0.05) than the other groups for approximately 4 h before recovering. The same trend was evident 1 h after p.m. feeding of

concentrate, where the pH of the 0 kg group was highest (P<0.05), intermediate (P<0.05) for the 4 kg group, and lowest (P<0.05) for the 8 kg group for a short period, after which the 4 kg group recovered having a similar (P>0.05) pH than that of the 0 kg group whereas the 8 kg group remained low (P<0.05). Throughout the night both the 4 kg and 8 kg group showed intermittent decreases (P<0.05) in pH compared with the 0 kg group.

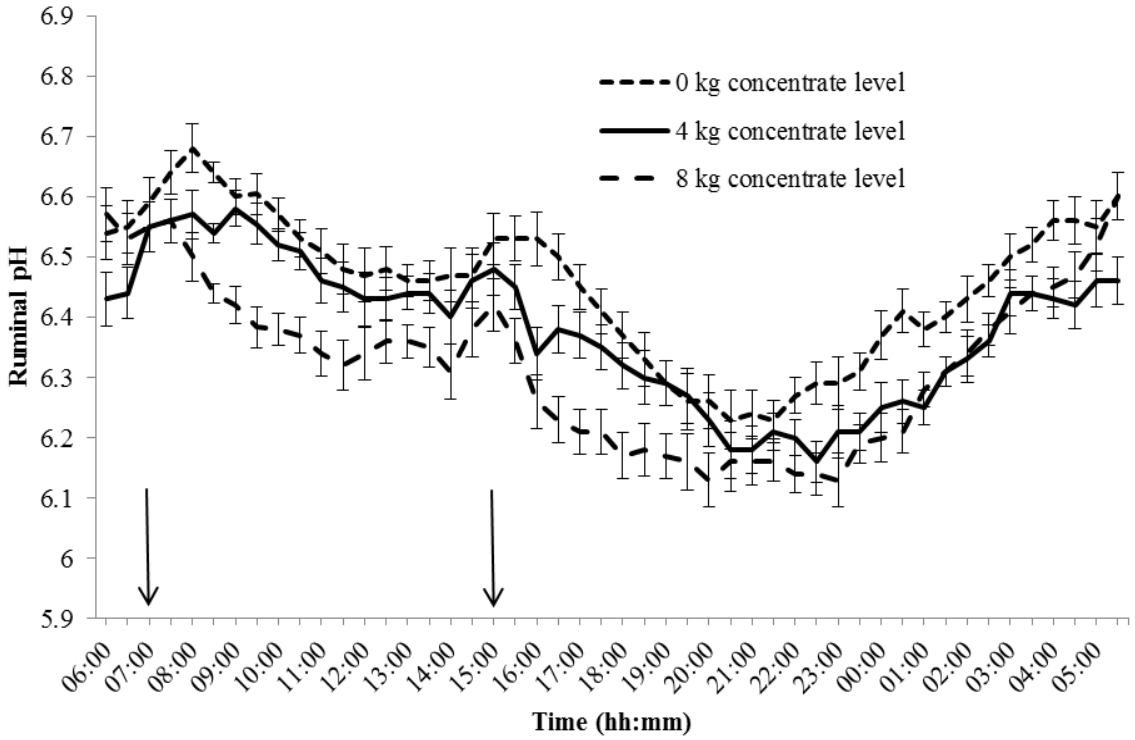

Figure 2-1 The effect of concentrate supplementation level on diurnal ruminal pH of early lactation Jersey cows grazing kikuyu-dominant pasture in late summer (n = 9). Error bars indicate standard error of mean and arrows indicate when concentrate was fed**.**

The treatment effect on mean ruminal pH, time spent below pH, VFA and NH_3-N concentrations, and DM and NDF disappearance are presented in Table 2-5. Diurnal ruminal pH and spot sample ruminal pH decreased linearly (P<0.05) with increasing concentrate feeding level. During both measurement periods, ruminal pH was lowest (P<0.05) for the 8 kg group compared with the 0 kg group, but similar to the 4 kg group. Time spent below pH of 6.6 tended to increase linearly with increasing concentrate feeding level.

Table 2-5 The effect of concentrate supplementation level on ruminal pH, volatile fatty acid and NH_3-N concentration, and *in sacco* pasture disappearance of early lactation Jersey cows grazing kikuyu-dominant pasture in late summer (mean of the rumen measurement periods; n = 9).

Item[1]	Concentrate level (kg/d as fed)			SEM[3]	P-value[4]		
	0	4	8		Con	Lin	Quad
Diurnal pH (over 72 h)	6.45[a]	6.38[ab]	6.32[b]	0.029	0.029	0.010	0.82
Spot sample pH	6.38[a]	6.25[ab]	6.16[b]	0.033	0.003	<0.001	0.70
Time below (h)							
pH 5.8	0.44	0.11	1.94	0.262	0.001	0.32	0.40
pH 6.0	1.67	1.33	3.39	0.693	0.13	0.32	0.43
pH 6.2	5.17	6.61	5.83	1.750	0.85	0.70	0.47
pH 6.4	10.9	12.6	13.2	2.889	0.85	0.37	0.81
pH 6.6	15.1	19.2	20.3	2.894	0.42	0.060	0.50
NH_3-N (mg/dL)	10.8	12.8	12.1	0.58	0.10	0.14	0.10
Total VFA (m*M*/L)	94.6	92.7	91.1	5.96	0.92	0.69	0.98
Acetic (m*M* %)	81.6	80.1	81.5	0.82	0.39	0.95	0.18
Propionic (m*M* %)	11.8	11.9	11.2	0.54	0.65	0.48	0.56
Butyric (m*M* %)	5.21[b]	6.65[a]	6.14[ab]	0.252	0.007	0.027	0.010
Isobutyric (m*M* %)	0.42	0.42	0.34	0.024	0.053	0.030	0.23
Valeric (m*M* %)	0.38	0.36	0.35	0.031	0.82	0.54	0.94
Isovaleric (m*M* %)	0.56[a]	0.53[ab]	0.43[b]	0.032	0.030	0.012	0.40
Caproic (m*M* %)	0.11	0.08	0.07	0.010	0.091	0.037	0.57
DM disappearance[2]							
6 h	0.28[a]	0.26[b]	0.26[b]	0.003	0.008	0.005	0.11
18 h	0.50	0.49	0.45	0.015	0.12	0.050	0.57
30 h	0.69[a]	0.66[a]	0.61[b]	0.009	<0.001	<0.001	0.27
NDF disappearance							
6 h	0.13[a]	0.11[ab]	0.09[b]	0.008	0.017	0.005	0.86
18 h	0.43	0.41	0.36	0.020	0.060	0.027	0.37
30 h	0.64[a]	0.62[a]	0.55[b]	0.013	0.002	<0.001	0.17
NDF k_d							
6 h	0.027[a]	0.022[ab]	0.018[b]	0.0016	0.012	0.004	0.82
18 h	0.045	0.044	0.035	0.0034	0.16	0.086	0.37
30 h	0.050[a]	0.047[a]	0.038[b]	0.0020	0.004	0.002	0.23
Mean	0.040[a]	0.038[a]	0.031[b]	0.0018	0.008	0.003	0.29

[a,b,c] Row means with different superscripts differ significantly at P<0.05.
[1] NH_3-N–ammonia nitrogen; VFA–volatile fatty acid; DM–dry matter; NDF–neutral detergent fibre; NDF k_d–rate of neutral detergent fibre disappearance.
[2] Coefficient.
[3] SEM–standard error of mean.
[4] Con–contrast; Lin–linear; Quad–quadratic.

Total VFA, acetic and propionic acid, and NH_3-N concentration were unaffected by treatment. Butyric acid increased linearly and quadratically ($P<0.05$) with increasing concentrate feeding level. The 8 kg group had a similar butyric acid concentration than both the 0 and 4 kg group, while the 4 kg group had a greater ($P=0.007$) butyric acid concentration than the 0 kg group. Both isobutyric and capronic acid concentration tended to decrease linearly ($P<0.10$) with increasing concentrate feeding level. Isovaleric acid concentration decreased linearly ($P=0.012$) with increasing concentrate feeding level. The 4 kg group had a similar isovaleric acid concentration than both the 0 and 8 kg group, while the 0 kg group had a greater ($P=0.030$) isovaleric acid concentration than the 8 kg group.

In sacco DM and NDF disappearance, and NDF k_d decreased linearly ($P<0.05$), irrespective of incubation period, with increasing concentrate feeding level. However, after 18 h of incubation DM disappearance and NDF k_d only tended to decrease linearly with increasing concentrate feeding level. After 6 h of ruminal incubation of the pasture: DM disappearance was similar for the 4 kg and 8 kg group but greater ($P=0.008$) for the 0 kg group; while both NDF disappearance and NDF k_d were greater ($P<0.05$) for the 0 kg group relative to the 8 kg group but remained unaffected for the 4 kg group. After 30 h of ruminal incubation: DM and NDF disappearance, and NDF k_d were similar for the 0 kg and 4 kg group but lowest ($P<0.05$) for the 8 kg group. The mean NDF k_d was similar the 0 kg and 4 kg group but lowest ($P=0.008$) for the 8 kg group.

2.5 DISCUSSION

Pasture-based dairy systems are inevitably subject to several challenges involving animal and system parameters, such as seasonal variation in pasture availability and nutritive quality, which can influence DMI and the milk response to concentrate supplementation (Roche et al., 2009). Adding to that, DMI has been labeled as the main driver for enteric CH_4 emissions (Ellis et al., 2007). In view of this, it is expected that enteric CH_4 emissions of cows will vary across seasons, therefore highlighting the significance of determining enteric CH_4 emissions from seasons other than spring; hence, promoting more accurate emissions per annum for pasture-based systems.

The present study was conducted in late summer reflecting a sub-tropical (kikuyu) and temperate (ryegrass) pasture mix with a similar chemical composition as the pasture offered by Bargo et al. (2002); as supported by the similar NDF intake as % of BW for the two studies. Previous studies testing the effect of concentrate level on enteric CH_4 emissions utilised mostly temperate pasture during spring with NDF values below 48%, CP values between 21 and 25%, and ME values between 11 and 12 MJ/kg of concentrate DM (Jiao et al. 2014; Muñoz et al., 2015). Individual pasture DMI as determined with the rising plate meter was very low (6 kg of DM/cow per day). This was plausibly due to the mixed sward causing a discrepancy in pre-grazing height hence giving rise to a misleading linear regression predicting pasture DM yield. Despite this, the target post-grazing height of 5.5 cm was achieved therefore indicating that the pasture was not over- or under-utilised (Fulkerson et al., 1999).

Between the two extreme treatments, pasture DMI decreased with increasing concentrate level while total DMI as well as GE intake increased, which is in agreement with Bargo et al. (2003). It was previously reported that concentrate supplementation in pasture-based systems reduced ruminal pH, increased total VFA concentration, reduced NH_3-N concentration, and when fed at high levels in corn-based form (>8 kg of DM/cow per day) it reduced the rate of pasture degradability (Bargo et al., 2002; Bargo et al., 2003). This was also observed in the present study except for total VFA and NH_3-N being unaffected by treatment. Pasture substitution (kg of pasture DMI/kg of concentrate DMI) that occurred within this study (0.56: 0 *vs.* 4 kg; 0.23: 4 *vs.* 8 kg; and 0.39: 0 *vs.* 8 kg concentrate treatment) was below average of previous reports (Bargo et al., 2003). Pasture substitution is less profound at low DHA than at high DHA (Bargo et al., 2002), as was seen here. The challenge of measuring accurate individual pasture DMI under grazing conditions is well known, even so it is essential when evaluating and expressing CH_4 emissions. Therefore, we decided on using an indirect marker (TiO_2) to account for between and within-animal variation, rather than using ME back calculations that does not account for this. The most recent grazing studies evaluating CH_4 emissions utilised the ME back calculation method to determine pasture DMI (Jiao et al. 2014; Muñoz et al., 2015).

An overall milk response of 1.38 and 1.20 kg of milk/kg of concentrate DMI were achieved moving from the 0 to 4 kg and 0 to 8 kg concentrate level, respectively, while a

marginal milk response (4 *vs.* 8 kg concentrate level) of 0.97 kg of milk/kg of concentrate DMI was achieved. This milk response is on the high end of previous published responses (Lovett et al., 2005; Jiao et al. 2014; Muñoz et al., 2015), owing to the lower than average substitution rate of the current study and that milk response was calculated relative to an unsupplemented treatment; hence, an above average milk response was expected. Bargo et al. (2003) confirmed in a review study that substitution rate is negatively related to milk response. The milk composition response observed within this study was largely as described by Bargo et al. (2003), where milk fat decreases while milk lactose increases with increasing concentrate level. The observed response in MUN was a result of the diluting effect imposed by the lower protein content of the concentrate fed relative to that of the pasture that was on offer. According to Seymour et al. (2005), DMI and milk production is positively related to propionic and butyric acid concentrations in the rumen, whereas milk fat content is positively related to acetic acid. The lack of a response in acetic and propionic acid as observed in the present study, unfortunately, failed to support the observed increase in milk yield and decrease in milk fat content, possibly owing to the similar NDF intakes as % of BW between treatments. However, the observed increase in butyric acid did support the observed increase in DMI and milk yield as concentrate level increased. The improved BW and BCS with increasing concentrate level reflect the increase in GE intake.

When comparing our enteric CH_4 results to previous studies utilising predominantly ryegrass pasture (Lovett et al., 2005; Jiao et al., 2014; Muñoz et al., 2015; van Wyngaard et al., 2018b), we found discrepancies in the response of enteric CH_4 emissions towards concentrate supplementation. This could possibly be ascribed to different experimental designs, pasture management in terms of pasture quality and DHA, and methods on determining DMI and CH_4 emissions. Average enteric CH_4 emissions of the current study (357 g/d) closely resemble that of Lovett et al. (2005) and Muñoz et al. (2015), being 373 and 355 g/d, respectively. Both of these authors also reported increased CH_4 production with increasing concentrate level up to 6 and 5 kg of concentrate/d, respectively. Other grazing studies reported much lower average enteric CH_4 emissions with no treatment response, being 277 and 294 g/d for Jiao et al. (2014) and van Wyngaard et al. (2018b), respectively. Both of these studies had a maximum concentrate level of 8 kg/d. The

majority of the studies also reported a reduction in CH_4 intensity with increasing concentrate feeding level, except for Muñoz et al. (2015). The reduced CH_4 yield reported in this study was also only reported by Jiao et al. (2014), highlighting the noted discrepancy among the different grazing studies.

Even though the CH_4 emissions measured in this study seem high relative to previous grazing studies, it fits the universal linear relationship between CH_4 production and DMI as developed by Charmley et al. (2016). Dairy cows from that study were mainly fed a 70:30 forage (pasture, pasture hay, pasture silage, or lucerne hay) to concentrate (barley, triticale, or wheat) diet; similar to the present study ranging from a 100% to a 54% pasture component of the diet. It has been established that diets containing a lower NDF:starch ratio will result in a lower CH_4 production (Moe and Tyrrell, 1979; Knapp et al., 2014). When transposed a higher NDF:starch ratio will result in a higher CH_4 production. Consequently, the higher CH_4 emissions of this study reflect the higher NDF content of the pasture offered in comparison with previous grazing studies. This is further supported by Lovett et al. (2015) who obtained similar CH_4 production values where cows were supplemented with a fibre-based concentrate while grazing a pasture mix high in NDF (50%) relative to previous grazing studies (<48%).

According to McAllister and Newbold (2008), a reduction in fibre intake, imposed by the addition of grain to a forage diet, reduces ruminal pH, affecting DM and NDF digestibility (as was seen in the current study) and favours propionate production rather than acetate in the rumen. In the current study, the decreasing tendency in NDF intake failed to increase propionate production even though ruminal pH and DM disappearance decreased linearly with increasing concentrate level. Perhaps the observed decrease in diurnal ruminal pH (from 6.45 to 6.32) was not enough to cause a major shift in the microbial population in favour of propionate production. This is also evident in the rather small observed decrease in DM and NDF disappearance. This is supported by Kolver and De Veth, (2002) who reported that the optimal range for ruminal fibre digestion on pasture systems is at a ruminal pH of >5.8. The observed increase in butyrate supports the observed increase in CH_4 production as concentrate level increases, as butyrate and acetate are precursors for CH_4 production (van Nevel and Demeyer, 1996). The potential of

ruminal VFA and pH to act as proxies for enteric CH_4 emissions is variable (Negussie et al., 2017).

It was previously established in a meta-analysis that Y*m* distinctly decreases when the grain component in a diet exceeds 35 to 40% inclusion dependent on the level of DMI (Sauvant and Giger-Reverdin, 2009). In agreement, the Y*m* of the current study tended to decrease when the concentrate component increased from 0 to 46%. This was also the case in the study of Jiao et al. (2014) when the concentrate component increased from 12 to 46% resulting in the decreased Y*m*. The observed Y*m* within this study (7.9 to 9.0%) is slightly higher than that reported by recent grazing studies (5.3 to 6.7%; Jiao et al., 2014; Muñoz et al., 2015). This could be attributed to the higher NDF content of the summer pasture grazed during this study relative to the spring pasture, with inherent lower NDF content, grazed during those studies. Lassey (2007) reported that ruminant livestock, across different production systems, has a typical Y*m* range of 4 to 10%. Recently, a relative high Y*m* value of 9.2%, similar to that of the current study was reported by Dall-Orsoletta et al. (2016) where dairy cows in mid to late lactation on a partial total mixed ration were allowed to graze Italian ryegrass for short periods. Furthermore, Y*m* values of ruminants fed diets containing tropical grass diets (such as kikuyu) can range between 8.4 to 11.4% (Kurihara et al., 1999; Tangjitwattanachai et al., 2015), but can also be as low as 4.9% (Noguera and Posada, 2017).

The results of the current study demonstrated that concentrate supplementation on medium quality summer pasture can reduce CH_4 yield and intensity, but increases CH_4 production. The observed change in the rumen environment caused by the increased starch:NDF ratio was not great enough to favour propionate production, but rather favourable for butyrate production.

2.6 CONCLUSIONS

Enteric CH_4 emissions were measured from lactating Jersey cows grazing high quality summer pasture under a restricted DHA supplemented with three levels of concentrate (0, 4 and 8 kg). Although enteric CH_4 production increased, CH_4 yield and intensity decreased with increasing concentrate level. Surprisingly, propionate production did not increase, but butyrate production increased with increasing concentrate level. Concentrate supplementation is a viable option as CH_4 mitigation strategy for dairy cows grazing pasture during the summer months. However, the impact of concentrate feeding on total GHG emissions and profitability should not be ignored. Furthermore, results from this study can be used for future meta-analysis studies in developing robust prediction equations; hence, improving GHG inventories.

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CHAPTER 3

Effect of concentrate level on enteric methane emissions, production performance and rumen fermentation of Jersey cows grazing ryegrass pasture during spring

3.1 ABSTRACT

Dietary supplementation has been well documented as an effective enteric methane (CH_4) mitigation strategy. However, limited studies have demonstrated the effect of concentrate level on enteric CH_4 emissions from grazing dairy cows, and to our knowledge none of these studies included a pasture-only diet or reported on rumen fermentation measures. Sixty multiparous (4.0±1.51 SD) Jersey cows, of which six were rumen-cannulated, were used in a randomised complete block design, and the cannulated cows were used in a separate replicated 3 × 3 Latin square design, to investigate the effect of concentrate supplementation (0, 4, and 8 kg/cow per day; as fed) on enteric CH_4 emissions, milk production, dry matter intake (DMI), and rumen fermentation of dairy cows grazing perennial ryegrass pasture during spring, following a 14-d adaptation period. The sulphur hexafluoride tracer gas technique was used to measure enteric CH_4 emissions from 10 cows of each treatment group over a single 9-d measurement period. Parallel with the CH_4 measurement period, pasture DMI was determined using TiO_2 and indigestible neutral detergent fibre as external and internal markers, respectively, while milk yield, milk composition, cow condition, and pasture pre- and post-grazing measurements were also recorded. Total DMI (13.4 to 18.0 kg/d), milk yield (12.9 to 19.2 kg/d), energy corrected milk (14.6 to 20.7 kg/d), milk lactose content (46.2 to 48.1 g/kg) and gross energy intake (239 to 316 MJ/d) increased, while milk fat content (50.0 to 44.2 g/kg) decreased with increasing concentrate feeding level. Volatile fatty acid concentrations and ruminal pH were mostly unaffected by treatment, while dry matter disappearance decreased and NH_3-N concentration increased with increasing concentrate feeding level. Methane production (258 to 302 g/d) and CH_4 yield (20.6 to 16.9 g/kg of DMI) were similar for all cows, while pasture DMI (13.4 to 10.8 kg/d) and CH_4 intensity (20.4 to 15.9 g of CH_4/kg of milk yield)

decreased linearly with increasing concentrate feeding level. Results indicate that concentrate supplementation on high quality pasture-only diets have the potential to effectively reduce CH_4 emissions per unit of milk yield from grazing cows during spring.

Key words: CH_4 measurement; perennial ryegrass; methane mitigation; pasture-based; SF_6

3.2 INTRODUCTION

Over the past decade, enhanced management and genetics in dairy farming have resulted in increased milk production which led to, *inter alia*, improved feed efficiency and a more cost-effective product (Negussie et al., 2017). Conversely, dairy farming results in emissions of methane (CH_4) gas that is mainly produced by microbes in the rumen. Methane is a damaging greenhouse gas with 28 times the greenhouse potential of carbon dioxide over a 100 year period (Myhre et al., 2013) and signifies a loss of energy that could have been converted into animal products. The livestock sector is a major contributor to the buildup of CH_4 emissions in the atmosphere. The South African cattle industry produced 964 Gg of CH_4 emissions during 2010, of which 13.5% was represented by the dairy sector mainly in the form of enteric CH_4 emissions (du Toit et al., 2013). The latter statistics were obtained by means of tier 2 methodologies as described by the IPCC (2006). The need to implement a more refined method, such as tier 3 methodologies, to further improve the accuracy of current national greenhouse gas inventories as well as the need to alleviate enteric CH_4 emissions has become a growing concern on an international level.

Several effective mitigation strategies for enteric CH_4 emissions have been extensively reviewed (Hristov et al., 2013; Knapp et al., 2014), which can be classified in the following categories: feeds and nutrition, rumen modifiers, and herd management and genetics. When selecting a mitigation strategy the combined effects of whole-farm profitability, on-farm practicality, and adoption potential should be considered (Hristov et al., 2013). Feeding high levels of concentrates as mitigation strategy meets the latter conditions. Tyrrell and Moe (1972) showed that CH_4 yield (g/kg of dry matter intake (DMI)) and intensity (g/kg of animal production) will decrease by increasing the proportion of concentrate in the diet if animal production remains the same or is increased. However, although concentrate feeding level has been evaluated extensively as a CH_4

mitigation strategy in confined dairy systems (Yan et al., 2010; Aguerre et al., 2011), pasture-based dairy systems received much less attention. The limited work undertaken has generally indicated that milk production and total DMI increased with increasing concentrate level, whereas the CH_4 emission response to treatment varied, with one study showing no treatment response (Young and Ferris, 2011). The level of concentrate evaluated in these limited studies ranged from 1 to 8 kg/cow per day and cows mainly grazed perennial ryegrass (*Lolium perenne*) dominant pasture during spring.

To our knowledge, no grazing study to date has examined the effect of concentrate level on enteric CH_4 emissions with the inclusion of a pasture-only treatment. Furthermore, although the potential of rumen parameters such as volatile fatty acids (VFA) and pH to act as proxies for enteric CH_4 emissions is variable (Negussie et al., 2017), CH_4 emissions studies that include these rumen fermentation measurements can be beneficial for future CH_4 proxy meta-analysis studies.

Thus, the aim of the study was to determine the effect of different concentrate levels (including a pasture-only treatment) on CH_4 emissions, production performance and rumen fermentation of Jersey cows grazing perennial ryegrass pasture during spring. We hypothesised that an increased concentrate level will increase milk production and total DMI while decreasing CH_4 yield and intensity. We further hypothesised that enteric CH_4 emissions will increase as total DMI increases. Results obtained from this study can be used to improve the accuracy of the greenhouse gas inventory of the pasture-based South African dairy sector, and may have application to grazing based dairy sectors in other countries.

3.3 MATERIALS AND METHODS

3.3.1 Location and Animal Ethical Clearance

The study was conducted during spring of 2015 (September - November) at the Outeniqua Research Farm (33°58´S, 22°25´E; altitude 210 m above sea level) which forms part of the Western Cape Department of Agriculture (Elsenburg, South Africa). The study area has a temperate climate with a long-term (45 years) mean annual precipitation of 732

mm, distributed throughout the year, and a mean daily maximum and minimum temperature range of 18°C to 25°C, and 7°C to 15°C, respectively. Ethical clearance for animal care and use was obtained from the Western Cape Department of Agriculture (Elsenburg, South Africa) before commencement of the study (DECRA approval number: R114/115).

3.3.2 Animals, Experimental Design and Treatment

Sixty multiparous Jersey cows (six rumen-cannulated) with mean pre-experimental milk yield of 20.1 (±2.29 SD) kg/d, 142 (±52 SD) days in milk (DIM), mean parity of 4.0 (±1.51 SD), and mean body weight of 398 (±33.2 SD) kg were selected from the Outeniqua dairy herd. Intact cows (54) formed part of a production study and were blocked (18 blocks) according to pre-experimental milk yield, DIM, and parity in one of three treatment groups. Each treatment group was then randomly assigned to one of three treatments that differed by level of concentrate feeding: 0, 4 and 8 kg/cow per day (as fed basis). Furthermore, the six rumen-cannulated cows (previously fitted with Bar Diamond #1C rumen cannulae; Bar Diamond Inc, Idaho, USA) formed part of a separate rumen study with a duplicated 3 × 3 Latin square design, which ran concurrent with the production study. Each of the rumen-cannulated cows was subjected to the three treatments over 20-d periods (14 d adaptation and 6 d data collection). Concentrate was fed individually to cows in pellet form split in two equal portions during milking. The ingredient composition of the concentrate offered was as follows (g/kg of dry matter; DM): 695 ground maize, 116 soybean oilcake, 34 sugarcane molasses, 20 limestone ($CaCO_3$), 3.7 monocalcium phosphate, 5.6 salt, 3.1 magnesium oxide and 1 trace mineral and vitamin premix (containing 4 mg of Cu/kg, 10 mg of Mn/kg, 20 mg of Zn/kg, 0.34 mg of I/kg, 0.2 mg of Co/kg, 0.06 mg of Se/kg, 6×10^6 IU of vitamin A/kg, 1×10^6 IU of vitamin D3/kg, and 8×10^3 IU of vitamin E/kg). Cows were allowed a 14-d dietary adaptation period, followed by a 52-d data collection period that commenced September 4 and ended October 26.

3.3.3 Pasture and Grazing Management

The experimental paddock (8.55 ha) was under permanent irrigation. The pasture consisted of perennial ryegrass (*Lolium perenne* L) (69%) kikuyu (*Pennisetum clandestinum*) (6%), white clover (*Trifolium repens*; 8%), other grass (*Lolium multiflorum* and *Paspalum dilatatum*; 16%), and broad-leaf weeds (1%). The soil type was characterised as a Podzol soil type (Swanepoel et al., 2013). The paddock was divided into strips (150 m x 15 m) which were top-dressed after each grazing with 42 kg of nitrogen/ha using limestone ammonium nitrate (containing 280 g of nitrogen/kg). Cows were held back after milking to allow simultaneous access to fresh pasture as one group, which was allocated twice daily after milking with grazing areas being back-fenced. A strict daily herbage allowance was implemented and was continuously adjusted throughout the study period, to ensure a target post-grazing height of 5.5 cm above ground level. This was attained by measuring pre- and post-grazing pasture height with a rising plate meter (Jenquip folding plate pasture meter; Jenquip, Feilding, NZ) by taking 100 readings in a zigzag pattern across the grazing area. Pasture yield above ground (pre- and post-grazing) were estimated using the following site and season specific linear regression equation: Pasture yield (kg of DM/ha) = [120 × pasture height (rising plate meter reading)] – 898 (R^2 = 0.75). Rising plate meter reading is defined in 0.5 cm units.

3.3.4 Measurements

3.3.4.1 Animal performance

Cows were milked twice daily (0530 and 1330 h) using a 20-point swing-over milking machine, and milk yield was automatically recorded with weigh-all electronic milk (Dairymaster, Causeway, Co. Kerry, Ireland). Milk fat, milk protein, milk lactose and milk urea nitrogen (MUN) were determined from composite morning and afternoon milk samples using a Milkoscan FT+ milk analyzer (FOSS Analytical, DK-3400 Hillerød, Denmark), while somatic cell count (SCC) was determined using a Fossomatic FC (FOSS Analytical). Energy corrected milk was calculated using the equations of Tyrrell and Reid (1965): ECM = milk yield (kg/d) × [milk energy content (MJ/kg)]/3.1; where, milk energy

content (MJ/kg) = [0.0384 × milk fat (g/kg)] + [0.0223 × milk protein (g/kg)] + [0.0199 × milk lactose (g/kg)] – 0.108. Fat-corrected milk (FCM), standarised at 4% milk fat content, was calculated using the equation of Gaines (1928): FCM = [0.4 × milk yield (kg/d)] + [15 × milk fat (kg/d)]. Milk data from the rumen-cannulated cows were excluded from the treatment group mean due to the experimental design.

Cow body weight and body condition score (BCS) were recorded, before afternoon milking, at the start and the end of the study. Body weight was electronically recorded over two consecutive days using a fixed weighing scale (Tru-Test EziWeigh v. 1.0 scale, 0.5 kg accuracy, Auckland, New Zealand) and BCS was determined using the 1 to 5 scale scoring system of Wildman et al. (1982).

3.3.4.2 Dry matter intake

Individual pasture DMI was estimated with the use of titanium dioxide (TiO_2) as an external marker to determine faecal output (FO) and indigestible neutral detergent fibre (iNDF) as an internal marker to determine forage digestibility. Ten cows (block 1 to 10) per treatment group were each dosed with 3 g of TiO_2 twice daily over the last 10 days of the experiment, with faecal samples collected twice daily over the last six days of the experiment (Pinares-Patiño et al., 2008). One additional cow per treatment was included for background TiO_2 analysis. Faecal samples were immediately oven dried (65°C, 72 h), pooled within-animal, milled to pass a 1 mm sieve, and analysed for TiO_2 concentration by the method of Myers et al. (2004). Faecal output was calculated from the daily TiO_2 dose and TiO_2 concentration in faeces according to de Souza et al. (2015).

Representative pasture samples were cut (approximately 3 cm aboveground level) daily during the DMI measurement period on the successive grazing-strips. Pasture samples were immediately oven dried (55°C, 72 h), pooled and milled to pass a 1 mm sieve. Concentrate, pasture and faecal samples were incubated *in situ* for 288 h in polyester bags (07-11/5 Sefar Petex cloth, Sefar AG, Heiden, Switserland) to determine iNDF (Krizsan et al., 2015). After incubation, neutral detergent fibre (NDF) concentration was determined according to Robertson and van Soest (1981) using an Ankom^{200} fibre analyser (Ankom Technology Corp., Fairport, NY) assayed with a heat-stable α-amylase (protein enzyme EC 3.2.1.1; 1,4-α-D-glucan glucanohydrolase) and anhydrous sodium sulfite, and

expressed inclusive of residual ash. Pasture DMI was calculated using the equation of Cabral et al. (2014): Pasture DMI (kg/d) = [[FO (kg/d) × iNDF faeces (kg/kg)] – iNDF concentrate intake (kg/d)]/iNDF forage (kg/kg).

3.3.4.3 Enteric methane emissions

Methane emissions from individual cows were measured using the sulphur hexafluoride tracer gas (SF_6) technique as described by O'Neill et al. (2011) for grazing dairy cows. This measurement was done concurrently with the faecal collection period of the DMI measurement using the same 30 cows. The CH_4 measurement period was over a maximum of nine consecutive days to enable collection of five samples representative of the complete daily emissions of gas from each cow. Empty permeation tubes (P&T Precision Engineering Ltd., Unit 2, Naas Industrial Estate, Naas, Co. Kildare, W91 KA4C, Ireland) were loaded with 3.0 (±0.19 SD) g of SF_6 gas during August 2015. The mean release rate of the permeation tubes was 6.43 (±0.40 SD) mg of SF_6/d and ranged from 5.48 to 7.07 mg of SF_6/d one week prior dosing. This was obtained by calibrating the filled tubes in a dry incubator (Labcon Incubator Model FS1M8, Ferndale, Johannesburg) set at 39.0°C for five weeks, weighing the tubes (Sartorius BP210S, Sartorius AG, Göttingen, Germany; 0.0001 g accuracy) every third morning to produce an 11-point regression curve (R^2 > 0.9995). The permeation tubes were blocked by release rate and randomly allocated to both experimental treatment and cow within treatment. Tubes were individually placed in a size 10 gelatin capsule (Torpac Inc., 333 Route 46, Fairfield, NJ 07004, USA) and dosed *per os* 7 d prior to the measurement period using a plastic capsule-dose-applicator.

Eructed gasses were continuously sampled over a 24-h period in cylindrical, back-mounted polyvinyl chloride (PVC) gas-collection canisters of 1700 mL with an initial sampling rate of approximately 0.54 mL/min. This sampling rate allowed for the evacuated canister to fill to approximately 45% over a 24 h sampling period. Canisters were mounted on the back of the cows with the technique of van Wyngaard et al. (2018), but without the bespoke shaping shaft. The current study supported the development of the back-mounted harness as described by van Wyngaard et al. (2018). Canisters were reused after flushing residue gas by evacuating to 98 kPa vacuum, filling with ultra-high purity nitrogen gas (999.99 g/kg) and evacuating again to 98 kPa vacuum, repeated five times. Stainless-steel

capillary tubes (1/16" OD x 0.2" ID; YY-RES-21503; LECO Co., Saint Joseph, MI 49085, USA) cut to 50 mm length and crimped using a table top vice-grip were used as flow restrictors.

Four field canisters were used to sample background (ambient) concentrations of SF_6 and CH_4. These background canisters were hung on the fence along each side of the grazing area where the cows were allocated. Background canisters were replaced every 24 h with evacuated canisters during the CH_4 measurement period. Only background canisters were used for this exercise and not sample canisters. Background gas concentrations from all canisters were averaged per day to give a single estimate for all experimental cows.

A piston sub-sampler (National Institute of Water and Atmosphere (NIWA) Ltd., Viaduct Harbour, Auckland Central, 1010, NZ) was used to extract and subsample the undiluted gas sample from the canister into three 12 mL glass vials (Labco Exetainer, Labco Ltd., Lampeter, Ceredigion, SA48 7HH, UK). Gas samples were analysed using an automated gas analyser equipped with a Gilson Sample Changer (Gilson, Inc., Middleton, WI 53562-0027, USA) modified at NIWA to analyse pressurised air samples in Labco Exetainers, and a GC equipped with a flame-ionisation detector and an electron-capture detector (Hewlett Packard Model 6890, Palo Alto, CA, USA). Separation of CH_4 and SF_6 was attained using two parallel configured Alltech Porapak-Q 80-100 mesh columns (3.6 m × 3 mm stainless steel; Grace Davison Discovery Sciences, Deerfield, IL, USA). The flame-ionisation detector operated at 250°C and the electron-capture detector at 400°C using ultra-high purity nitrogen gas and argon as majority gas with 10% CH_4 added as carrier gasses (30 mL/min flow), respectively. Sample loops were flushed away from the flame-ionisation detector so the CH_4 in the electron-capture detector carrier gas was not carried through to the flame-ionisation detector. A suite of three standards of SF_6 and CH_4 mixtures from NIWA were associated with the analyses of each batch. Methane production (g/d) was calculated using equation 2 from the study of Williams et al. (2011).

3.3.4.4 Rumen fermentation

Six rumen-cannulated cows were used in the rumen fermentation study during each 20-d sampling period. Indwelling TruTrack pH Data Loggers (Model pH-HR mark 4, Intech Instruments Ltd., Riccarton, Christchurch 8011, NZ), attached to the rumen cannula,

were used to log diurnal pH patterns over a 72 h period (10 min frequency). Buffer solutions of pH 4 and 9 were used to calibrate the loggers and buffer solution of pH 7 was used as conformant. Logger drift was tested by placing the calibrated loggers in distilled water for 18 h where pH was monitored with a calibrated handheld pH logger (pH340i pH meter/data logger attached with a Sentix 41 pH electrode; WTW, 82362 Weilheim, Germany). A manual vacuum pump was used to collect ruminal fluid (100 mL) at 8 h intervals (0600, 1400 and 2200 h) from the ventral sac of each cow. Ruminal pH was immediately measured after sampling with the handheld pH logger (spot sample pH), and successively filtered through cheesecloth (four layers), subsampled in airtight containers and frozen for subsequent NH_3-N (Broderick and Kang, 1980) and VFA (Filípek and Dvořák, 2009) analysis. The nylon bag procedure of Cruywagen (2006) was used to determine the *in sacco* DM disappearances of the grazed pasture after 6, 18 and 30 h incubation periods.

3.3.5 Feed Sampling and Analysis

Representative concentrate and pasture samples (one pasture sample consisted of six pooled pasture samples cut approximately 3 cm above ground level from the successive grazing-strip) were collected weekly, dried at 55°C for 72 h (initial DM), ground to pass through a 1 mm sieve (SMC hammer mill), and analysed for DM, ash and CP (nitrogen content determined using a LECO Trumac^{TM} N Determinator, LECO Corporation, Saint Joseph, MI, USA) according to procedures of AOAC (2000; methods 934.01, 942.05, and 968.06, respectively). Samples were also analysed for NDF content, as described before, gross energy (GE; MC–1000 modular calorimeter, operator's manual), mineral composition (AgriLASA, 1998; method 6.1.1), and *in vitro* organic matter digestibility (OMD) according to Tilley and Terry (1963) using rumen fluid from a rumen-cannulated SA Mutton Merino ram fed good-quality lucern hay. Metabolisable energy (ME) was calculated using the equations of MAFF (1984): $ME_{concentrate}$ = 0.84 (GE × OMD), and $ME_{pasture}$ = 0.81 (GE × OMD).

3.3.6 Statistical Analysis

Milk yield (including FCM and ECM), milk composition, bodyweight change and body condition parameters (18 blocks) over the course of the study and for the duration of the DMI and CH_4 measurement period along with DMI and CH_4 emissions parameters (10 blocks) were analysed as a randomised complete block design with ANOVA to test for differences between treatment effects. The residuals were acceptably normal with homogeneous treatment variances, except for SCC which were log (base 10) transformed. Covariate analysis was not significant, with pre-experimental milk yield, DIM and parity as covariates; hence, excluded from the statistical analysis.

For the rumen fermentation study (ruminal pH parameters, fermentation end-products and *in sacco* DM disappearances) a replicated 3 × 3 Latin square design was implemented to test for differences between treatment effects. Time spent below ruminal pH of 6.6, 6.4, 6.2, 6.0, and 5.8 was Poisson distributed and thus analysed with generalised linear model analysis to test for differences between treatment effects.

Treatment means were compared using Tukey's least significant difference test at the 5% level of significance (Snedecor and Cochran, 1980). Data were analysed using the statistical program GenStat (Payne et al., 2014).

Daily CH_4 emissions of individual cows were averaged to yield a single daily value for each cow representative of the entire sampling period. The modified Z-score was used to identify outlying CH_4 data. Data associated with 'modified Z-scores' of >3.5 (absolute value) were labelled as outliers (Berndt et al., 2014). A 71% successful collection rate was achieved from the 217 gas samples collected. The remainder was lost due to blockages in the capillary flow restrictor and broken sampling lines during the 24-h collection periods.

3.4 RESULTS

3.4.1 Feed Composition and Pasture Management

The chemical composition of the dairy concentrate and pasture offered averaged across the 7-wk study period is presented in Table 3-1. Cows were offered a daily herbage allowance of 12.2 kg of DM/cow per day, 3 cm above ground level, and the average pasture yield was 1.9 t of DM/ha (Table 3-2). The target post-grazing pasture height was 5.5 cm, but the mean measured post-grazing height was 5.85 cm. According to the rising plate meter measurements, cows consumed approximately 73% of the offered daily herbage allowance.

Table 3-1 Chemical composition (mean ± SD) of the concentrate and of the pasture offered averaged across the 7-wk study period.

Item[1]	Concentrate (n = 7)	Pasture[2] (n = 5)
Initial DM (%)	89.9 ± 2.99	13.1 ± 11.8
DM composition (g/kg of DM or as stated)		
CP	132 ± 2.2	195 ± 21.9
NDF	92.8 ± 1.89	493 ± 24.7
Ash	65 ± 0.8	110 ± 5.9
*IV*OMD	933 ± 30.3	867 ± 40.0
GE (MJ/kg of DM)	17.3 ± 0.05	17.8 ± 0.28
ME (MJ/kg of DM)	13.6 ± 0.47	12.5 ± 0.61
Mineral composition (g/kg of DM or as stated)		
Ca	12.2 ± 0.40	4.90 ± 0.190
P	4.98 ± 0.093	4.71 ± 0.309
Mg	3.91 ± 0.066	3.22 ± 0.169
K	9.52 ± 0.199	25.8 ± 3.89
Na	2.59 ± 0.077	18.6 ± 4.41
Mn (mg/kg of DM)	93.8 ± 6.08	53.9 ± 12.55
Cu (mg/kg of DM)	32.5 ± 4.02	8.84 ± 1.439
Fe (mg/kg of DM)	197 ± 8.8	155 ± 27.9
Zn (mg/kg of DM)	166 ± 10.9	49.6 ± 3.99

[1] DM–dry matter; CP–crude protein; NDF–neutral detergent fibre; *IV*OMD–*in vitro* organic matter digestibility; GE–gross energy; ME–metabolisable energy.
[2] Pasture–perennial ryegrass (*Lolium perenne*) dominant.

Table 3-2 Pre- and post-grazing measurements of the experimental ryegrass pasture averaged (mean ± SD) across the 7-wk study period.

Item[1]	7-wk study (n = 65)
Pasture height (cm)	
Pre-grazing	11.5 ± 1.52
Post-grazing	5.85 ± 0.61
Pasture yield (kg of DM/ha)[2]	
Pre-grazing	1865 ± 364
Post-grazing	504 ± 147
DHA (kg of DM/cow per day)	12.2 ± 1.67
Daily grazed area (m^2/cow)	66.8 ± 9.33
Pasture removed (kg of DM/cow per day)	8.90 ± 1.24

[1] DM–dry matter; DHA–daily herbage allowance.
[2] Pasture yield (kg of DM/ha) = (120 × rising plate meter height reading) – 898; estimated 3 cm aboveground level using a rising plate meter.

3.4.2 Milk Yield, Milk Composition and Cow Condition

Milk yield, FCM and ECM increased linearly (P<0.001) with increasing level of dairy concentrate (Table 3-3). Milk composition was unaffected by treatment, except for MUN that decreased linearly (P<0.001) stepwise with increasing concentrate level, milk protein that increased linearly (P=0.027), and SCC that decreased linearly (P=0.021) with concentrate supplementation. Despite this, milk fat yield, protein yield, and lactose yield increased linearly (P<0.001) with increasing level of dairy concentrate due to the observed increase in milk yield. Milk fat yield was higher (P<0.001) for cows receiving concentrate, irrespective of concentrate feeding level, compared with cows on the pasture-only diet. Change in BCS increased linearly (P=0.020) with increasing concentrate level.

Table 3-3 The effect of concentrate feeding level on milk production and cow condition of early lactation Jersey cows grazing perennial ryegrass pasture in spring during the 7-wk study.

Number of cows	18	18	18			
Item[1]	Concentrate level (kg/d as fed)			SEM[2]	P-value	
	0	4	8		Contrast	Linear
Milk yield (kg/d)	12.6[c]	17.1[b]	19.1[a]	0.42	<0.001	<0.001
FCM yield (kg/d)	14.0[c]	19.0[b]	20.7[a]	0.46	<0.001	<0.001
ECM yield (kg/d)	13.8[c]	19.0[b]	20.8[a]	0.47	<0.001	<0.001
Milk fat (g/kg)	47.5	47.7	45.8	0.78	0.18	0.13
Milk protein (g/kg)	35.2	36.3	36.4	0.37	0.047	0.027
Milk lactose (g/kg)	46.3	46.7	46.5	0.23	0.46	0.43
Milk solids (g/kg)	129	131	129	1.0	0.28	0.91
MUN (mg/dL)	13.6[a]	11.6[b]	9.21[c]	0.283	<0.001	<0.001
Log_{10} SCC	2.23	2.22	1.94	0.084	0.031	0.021
Milk fat yield (kg/d)	0.60[b]	0.81[a]	0.87[a]	0.021	<0.001	<0.001
Milk protein yield (kg/d)	0.44[c]	0.62[b]	0.69[a]	0.015	<0.001	<0.001
Milk lactose yield (kg/d)	0.58[c]	0.80[b]	0.89[a]	0.022	<0.001	<0.001
BW change (kg)	-1.28	+4.44	+6.44	2.900	0.16	0.17
BCS change (scale 1 to 5)	+0.03	+0.10	+0.17	0.040	0.065	0.020

[a,b,c] Row means with different superscripts differ significantly at P<0.05.
[1] FCM–4% fat corrected milk; ECM–energy corrected milk; Milk solids = milk fat + milk protein + milk lactose; MUN–milk urea nitrogen; SCC–somatic cell count; BW–body weight; BCS–body condition score.
[2] SEM–standard error of mean.

3.4.3 Dry Matter Intake and Enteric Methane Emissions

Faecal output was unaffected (P>0.05) by treatment, whereas pasture DMI decreased linearly (P=0.034) and total DMI increased linearly (P<0.001) with increasing concentrate feeding level (Table 3-4). Total DMI was the highest for both the 4 and 8 kg groups while being the lowest (P=0.003) for the 0 kg group. Furthermore, total DMI per kg bodyweight, GE intake and ME intake increased linearly (P<0.05) with increasing concentrate feeding level. Cows fed the 8 kg concentrate level had a higher (P=0.004) total DMI per kg bodyweight and a higher (P=0.005) GE intake compared with those fed the 0 kg level, but similar (P>0.05) to those fed the 4 kg level. Furthermore, cows fed the 4 and 8 kg concentrate level had similar (P>0.05) ME intakes, but higher (P<0.001) than those on the pasture-only diet. In contrast, NDF intake per kg bodyweight was not affected (P>0.05) by treatment. Individual CP intake tended to increase linearly (P=0.068) with increasing concentrate feeding level. Methane production (g/d) and CH_4 energy (MJ/d) tended to

increase (P=0.107) with concentrate supplementation. It was also observed that CH_4 intensity, in the form of g/kg of milk yield decreased linearly (P=0.031) and tended to decrease (P=0.088) in the form of g/kg of ECM with increasing concentrate feeding level. Methane yield (g/d) and CH_4 intensity in the form of g/kg of FCM, were unaffected (P>0.05) by concentrate supplementation.

Table 3-4 The effect of concentrate feeding level on dry matter intake, methane emissions and milk production of early lactation Jersey cows grazing perennial ryegrass pasture in spring during the methane measurement period.

Number of cows	10	10	10			
Item[1]	Concentrate level (kg/d as fed)			SEM[2]	P-value	
	0	4	8		Contrast	Linear
BW (kg)	414	402	395	11.7	0.52	0.27
Faecal output (kg of DM/d)	2.21	2.47	2.42	0.14	0.40	0.31
Intake						
Pasture DMI (kg/d)	13.4	12.8	10.8	0.81	0.082	0.034
Total DMI (kg/d)	13.4[b]	16.4[a]	18.0[a]	0.81	0.003	<0.001
NDF intake as % of BW	1.63	1.66	1.53	0.113	0.67	0.54
DMI as % of BW	3.30[b]	4.11[ab]	4.57[a]	0.236	0.004	0.001
GEI (MJ/d)	239[b]	290[ab]	316[a]	14.5	0.005	0.001
MEI (MJ/d)	168[b]	209[a]	233[a]	10.2	<0.001	<0.001
CP intake (kg/d)	2.62	2.97	3.05	0.16	0.15	0.068
CH_4 emissions						
CH_4 production (g/d)	258	321	302	20.0	0.107	0.15
CH_4/DMI (g/kg)	20.6	19.6	16.9	1.86	0.37	0.18
CH_4/milk yield (g/kg)	20.4	19.8	15.9	1.36	0.063	0.031
CH_4/ECM (g/kg)	17.9	17.4	14.6	1.28	0.18	0.088
CH_4/FCM (g/kg)	17.7	17.3	14.9	1.30	0.30	0.16
CH_4 energy (MJ/d)	14.3	17.7	16.7	1.10	0.107	0.15
Y*m* (%)	6.38	6.12	5.30	0.580	0.41	0.20
Milk yield (kg/d)	12.9[c]	16.7[b]	19.2[a]	0.40	<0.001	<0.001
FCM (kg/d)	14.8[b]	19.0[a]	20.3[a]	0.44	<0.001	<0.001
ECM (kg/d)	14.6[c]	18.9[b]	20.7[a]	0.45	<0.001	<0.001
Milk fat (g/kg)	50.0[a]	49.4[a]	44.2[b]	1.34	0.013	0.007
Milk protein (g/kg)	35.8	36.5	36.8	0.49	0.30	0.14
Milk lactose (g/kg)	46.2[b]	46.6[b]	48.1[a]	0.39	0.008	0.003

[a,b,c] Row means with different superscripts differ significantly at P<0.05.
[1] BW–body weight; FO–faecal output; DM–dry matter; DMI–dry matter intake; NDF–neutral detergent fibre; GEI–gross energy intake; MEI–metabolisable energy intake; CH_4–methane; ECM–energy-corrected milk; FCM–fat-corrected milk; CH_4 energy = (55.22 MJ*CH_4 g/d)/1000; Y*m*– CH_4 energy per gross energy intake.
[2] SEM–standard error of mean.

The effect of concentrate level on milk production and milk composition recorded during the CH_4 measurement period are presented in Table 3-4. Milk yield, FCM and ECM obtained during the CH_4 measurement period reflected the same trend as that of the 7-wk study period (Table 3-3), by increasing linearly ($P<0.001$) with increasing concentrate level. The treatment effect on FCM observed during the CH_4 measurement period did not increase stepwise with increasing concentrate level, as in the case of the 7-wk study period, but exhibited only an increase ($P<0.001$) for cows receiving concentrate, irrespective of concentrate level. Furthermore, milk protein content did not differ, whereas milk fat content decreased linearly ($P=0.007$) while milk lactose content increased linearly ($P=0.003$) with increasing concentrate feeding level, which was not the case during the 7-wk study period (Table 3-3). Milk fat content was higher ($P=0.013$) for cows on both the 0 and 4 kg than those on the 8 kg concentrate level. Cows in the 8 kg group had a higher ($P=0.008$) milk lactose content compared to the other treatment groups.

3.4.4 Rumen Fermentation

The effect of concentrate feeding level on diurnal ruminal pH, as recorded by the indwelling pH logging system, is depicted in Figure 3-1. It was noticeable that ruminal pH of cows fed the 8 kg concentrate level decreased ($P<0.05$) 1 h after receiving the morning concentrate and remained lower ($P<0.05$) than the other groups for approximately 2.5 h before recovering. Subsequently, 1 h after cows received the afternoon concentrate, ruminal pH of the 4 and 8 kg group decreased ($P<0.05$) and remained lower than the 0 kg group for 30 min, where after the pH of the 8 kg group decreased even lower ($P<0.05$) than that of the 4 kg group. This continued for 1 h before the pH of the 4 kg group recovered ($P>0.05$) to that of the 0 kg group while the pH of the 8 kg group remained the lowest ($P<0.05$) for an additional hour. During the course of the evening and early morning cows on the 4 kg and 8 kg concentrate level showed intermittent decreases ($P<0.05$) in pH compared with the 0 kg group. Mean diurnal ruminal pH (averaged over 72 h) tended to decrease linearly ($P=0.082$) with increasing concentrate feeding level (Table 3-5). Furthermore, a linear increasing trend ($P=0.079$) was evident in time spent below ruminal pH of 6.2 as concentrate feeding level increased. Ruminal NH_3-N concentration increased

linearly (P=0.007) with increasing concentrate feeding level, with cows fed concentrate, irrespective of feeding level, having a greater (P=0.002) ruminal NH_3-N concentration than cows on the pasture-only diet. Total VFA concentration was unaffected by treatment, however isobutyric acid tended to increase (P=0.089) with increasing concentrate feeding level. Pasture *in sacco* DM disappearance, after 6, 18 and 30 h incubation, decreased linearly (P<0.05) with increasing concentrate feeding level. The pasture-only group had a higher (P=0.006) *in sacco* DM disappearance than the 4 kg and 8 kg group after 18 h incubation, but only higher (P<0.05) than the 8 kg group after 6 h and 30 h incubation.

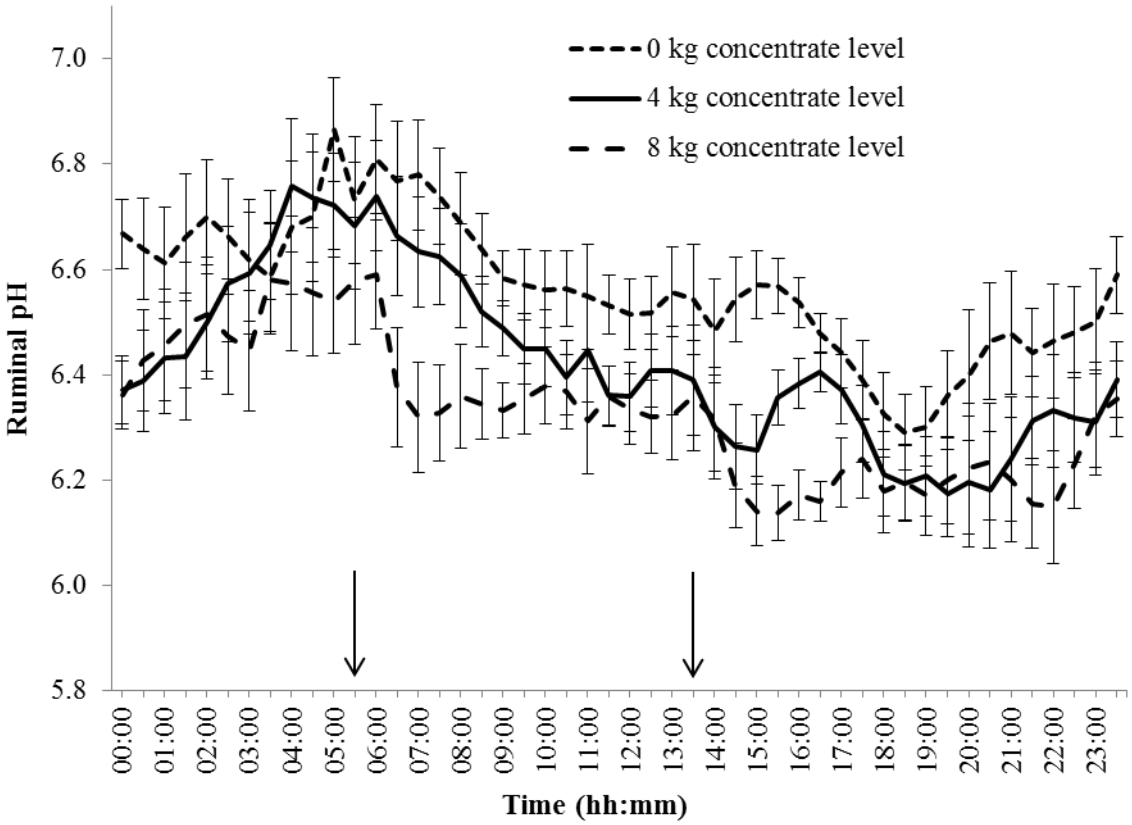

Figure 3-1 The effect of concentrate supplementation level (as fed) on diurnal ruminal pH of early lactation Jersey cows grazing perennial ryegrass pasture during spring (n = 6). Error bars indicate standard error of mean and arrows indicate when concentrate was fed.

Table 3-5 The effect of concentrate supplementation level on ruminal pH, volatile fatty acid profile, NH_3-N concentration, and dry matter disappearance of early lactation Jersey cows grazing perennial ryegrass pasture in spring (mean of the rumen measurement periods)**.**

Number of cows	6	6	6			
Item[1]	Concentrate level (kg/d as fed)			SEM[2]	P-value	
	0	4	8		Contrast	Linear
Diurnal pH (over 72 h)	6.57	6.39	6.33	0.075	0.17	0.082
Spot sample pH	6.22	6.13	6.09	0.046	0.23	0.11
Time below (h)						
pH 5.8	0.00	0.57	0.08	0.213	0.22	0.79
pH 6.0	0.42	2.50	2.58	1.010	0.31	0.19
pH 6.2	3.20	6.80	9.20	1.950	0.18	0.079
pH 6.4	8.90	11.2	13.4	2.350	0.46	0.23
pH 6.6	14.3	15.5	20.4	3.13	0.41	0.23
NH_3-N (mg/dL)	6.35[b]	13.0[a]	10.4[a]	0.713	0.002	0.007
Total VFA (m*M*/L)	91.8	91.5	91.7	4.68	0.99	1.00
Acetic (m*M* %)	65.3	65.6	65.1	0.68	0.88	0.87
Propionic (m*M* %)	18.1	18.2	18.6	0.53	0.80	0.54
Acetic to Propionic ratio	3.62	3.63	3.52	0.133	0.80	0.59
Butyric (m*M* %)	13.5	13.1	13.0	0.28	0.48	0.26
Isobutyric (m*M* %)	0.90	0.86	0.97	0.027	0.089	0.12
Valeric (m*M* %)	1.07	1.05	1.07	0.031	0.92	1.00
Isovaleric (m*M* %)	1.03	0.99	1.08	0.063	0.66	0.61
Caproic (m*M* %)	0.22	0.25	0.27	0.019	0.29	0.14
DM disappearance (coefficient)						
6 h	0.41[a]	0.38[ab]	0.36[b]	0.011	0.038	0.014
18 h	0.67[a]	0.64[b]	0.62[b]	0.012	0.006	<0.001
30 h	0.85[a]	0.83[ab]	0.80[b]	0.010	0.022	0.008

[a,b,c] Row means with different superscripts differ significantly at P<0.05.
[1] NH_3-N–ammonia nitrogen; VFA–volatile fatty acid; DM–dry matter.
[2] SEM–standard error of mean.

3.5 DISCUSSION

This study aimed to compare early lactation dairy cows grazing perennial ryegrass pasture during spring on the basis of DMI, milk production, rumen fermentation and CH_4 emissions; one group received zero concentrate, the second group received 4 kg (as fed) of concentrate, whereas the third group received 8 kg (as fed) of concentrate.

Pasture grazed in this study was comparable, in terms of botanical composition and quality, to that of pasture, one year after perennial ryegrass establishment, as reported by van der Colf et al. (2015), and also closely resembled the pasture quality of previous grazing studies that evaluated the effect of concentrate level on CH_4 emissions (Jiao et al., 2014; Muñoz et al., 2015). In addition, the quality of this pasture was of excellent standard (OMD>81%) which could result in a higher pasture DMI when compared with pasture having a lower OMD (Peyraud and Delagarde, 2013). The pre-grazing pasture yield or pasture mass in the current study (1865 kg of DM/ha) is within the range of previous grazing studies (1000 to 3800 kg of DM/ha) as summarised in a meta-analysis evaluating the effect of pre-grazing pasture mass on several different dairy cow production parameters (Pérez-Prieto and Delagarde, 2012). Pasture DMI (kg/cow per day) as determined with the rising plate meter was 28% (8.9 *vs*. 12.3) lower than the pasture DMI averaged across the treatments as determined with TiO_2 and iNDF. This discrepancy shows that pasture DMI estimated by both the TiO_2/NDF method and by the rising plate meter method should be interpreted with caution. Furthermore, we observed that pasture DMI decreased linearly with increasing concentrate level, indicating that a certain degree of pasture substitution was evident. Substitution rate is influenced by several pasture, animal and supplement factors, with pasture yield, daily herbage allowance and pasture quality (OMD) being identified as the most important pasture-related-factors (Bargo et al., 2003). In the current study the substitution rate (kg of pasture DMI/kg of concentrate DMI), calculated relative to the pasture-only treatment, was 0.15 and 0.33 for the 4 kg and 8 kg concentrate group, respectively, and were in agreement with previous grazing studies as reported by Bargo et al. (2003). Additionally, substitution rate is negatively correlated to milk response (Stockdale, 2000), as was seen here where the milk response (kg of milk/kg of concentrate) decreased as the concentrate level and substitution rate increased during the CH_4 measurement period; 1.06 and 0.88 increasing from the 0 to 4 kg and 0 to 8 kg of concentrate level, respectively, while a marginal milk response of 0.70 was attained when comparing the 4 kg to the 8 kg concentrate levels.

From a meta-analysis that included 211 concentrate supplementation studies using lactating dairy cows, Huhtanen and Hetta (2012) reported marginal positive responses between concentrate DMI and total DMI, milk yield, ECM yield, and milk protein and

milk lactose content, and marginal negative responses between concentrate DMI and forage DMI, and milk fat content. Similar responses were observed in our study during the CH_4 measurement period, except for milk protein content that remained unchanged by concentrate feeding level in agreement with previously published grazing studies evaluating the effect of concentrate level on CH_4 emissions and milk production responses (Lovett et al., 2005; Muñoz et al., 2015). This response reflects the decreasing marginal CP intake with increasing concentrate feeding level. Furthermore, Roseler et al. (1993) stated that MUN decreases as the diet CP:ME ratio decreases, as was evident in the current study where the diet CP:ME ratio decreased from 1.56 to 1.32 changing from the 0 kg to the 8 kg treatment as a result of the observed increase in energy intake as concentrate level increased.

Rumen fermentation parameters such as VFA concentration, pH, disappearance coefficients and NH_3-N can act, in some instances, as marginal proxies for milk production responses to feed alterations such as concentrate feeding level (Bargo et al., 2003). In the present study concentrate level did not impact biologically significant on the VFA profile and ruminal pH, however DM disappearance and NH_3-N concentration were affected by concentrate supplementation. The decrease in DM disappearance with increasing concentrate feeding level was also reported by Bargo et al. (2013), however the increase in NH_3-N concentration with increasing concentrate feeding level is in contrast with the findings of Bargo et al. (2003). In the current study, the increased NH_3-N concentration is supported by the observed increasing trend in CP intake towards increasing concentrate feeding level, which could lead to an increase in ruminally degradable CP. Additionally, this indicates that the pasture in the current study should have a lower CP content or ruminally degradable CP content than the pasture evaluated in the review study of Bargo et al. (2003). This discrepancy reflects the complexity of the relationship between concentrate level and rumen fermentation patterns on pasture-based systems. Regardless, the recurrent pattern of the diurnal ruminal pH variation around concentrate feeding time, as observed in the current study, is in agreement with Bargo et al. (2002) who reported that ruminal pH is the highest pre-concentrate feeding and lowest post-concentrate feeding.

Feeding high levels of concentrates has been identified as an effective enteric CH_4 mitigation strategy for cattle (Hristov et al., 2013; Knapp et al., 2014), albeit there are

limited studies that have evaluated the effect of concentrate feeding level on enteric CH_4 emissions from grazing dairy cows. Lovett et al. (2005) reported an increase in CH_4 emissions (346 *vs*. 399 g/d) and a tendency for decreased CH_4 emissions per kilogram fat corrected milk (FCM; 21.0 *vs*. 17.7 g/kg), while Jiao et al. (2014) reported a decrease in CH_4 emissions per kilogram energy corrected milk (ECM; 14.1 to 11.1 g/kg), per kilogram milk yield (15.4 to 10.8 g/kg), and per kilogram DMI (20.0 to 18.1 g/kg) when the concentrate level increased from 1 to 6 kg (as fed), and increased in 2 kg increments from 2 to 8 kg (as fed), respectively. In another study when concentrate level increased from 1 to 5 kg (as fed), CH_4 emissions (323 *vs*. 357 g/d for period 1, and 349 *vs*. 390 g/d for period 2) increased with increasing concentrate level (Muñoz et al., 2015). This discrepancy in the response of CH_4 emissions to concentrate feeding level can be attributed to different pasture DMI responses (as affected by several factors including daily herbage allowance and pasture substitution rate), method of estimating DMI and CH_4 emissions, and the statistical power of the experimental design.

When comparing our results to these limited grazing studies, we found that the average CH_4 emissions in the current study (294 *vs*. 277 g/d) closely resembles that of Jiao et al. (2014), who also fed a maximum concentrate level of 8 kg/d, but to Holstein-Friesians, while also reporting no treatment effect on CH_4 emissions (g/d). In the latter study, a pasture substitution rate of 0.73 was evident between the two extreme concentrate levels (2 and 8 kg/d), compared with 0.50 in the current study. This difference in substitution rate, most probably, led to the observed decrease in pasture DMI in the study of Jiao et al. (2014), whilst not in the current study. Additionally, the pasture-only group in the current study produced similar CH_4 emissions to that of the pasture-only group (258 *vs*. 251 g/d; 20.6 *vs*. 18.1 g/kg of DMI; 6.4 *vs*. 5.7% CH_4 energy per GEI (Y*m*), respectively) in a study of O'Neill et al. (2011), where the authors compared CH_4 emissions from Holstein-Friesian cows on a pasture-only diet (100% *Lolium perenne* L.) to cows on a total mixed ration diet. On the contrary, other grazing studies that evaluated the effect of concentrate feeding level on CH_4 emissions yielded greater average CH_4 emissions (294 *vs*. 372, and 355; Lovett et al. 2005, and Muñoz et al., 2015, respectively), compared with the current study. This could possibly be attributed to the greater feed intakes observed in those studies. The average CH_4 yield (19.0 g/kg of DMI) was similar to average values

reported in previous grazing studies, all of which implemented the SF_6 technique to measure CH_4 emissions: 18.7 (Lovett et al., 2005); 19.2 (O'Neill et al., 2011), 18.8 (Jiao et al., 2014), and 19.2 (Muñoz et al., 2015). Whereas, the average CH_4 intensity (18.7 g/kg of milk yield) was greater than that reported by Jiao et al. (2014) and Muñoz et al. (2015), 12.6 and 13.6, respectively, it was more closely related to the value of 19.4 as reported by Lovett et al. (2005). This difference can be ascribed to the greater milk production of the Holstein-Friesian cows in the studies of Jiao et al. (2014) and Muñoz et al. (2015), compared with that of Jersey cows (NRC, 2001). Whereas the similarity can be ascribed to the high fibre diet, induced by the fibre-based concentrate and pasture species present in the study of Lovett et al. (2005), that has been reported to reduce milk production (Bargo et al., 2003). The lack of a linear response in CH_4 yield and intensity (g/kg of ECM) was in agreement with Muñoz et al. (2015). These authors attributed their CH_4 intensity results to their milk response of 0.6 kg of milk/kg of concentrate (1 and 5 kg concentrate level), being the threshold for dilution of maintenance requirements over greater milk production units that could be a mechanism for reducing CH_4 intensity. Other factors as parity, DIM, breed, and pasture botanical composition and quality should not be ignored while interpreting enteric CH_4 emissions from grazing studies as all these factors, and more, can influence enteric CH_4 emissions from dairy cows (Muñoz et al., 2015).

When interpreting the VFA and pH results in relation to the CH_4 emission results obtained in this study, the observed similar CH_4 emissions between treatments can be explained, in part, by the similar acetic to propionic acid ratio and ruminal pH that were also observed between treatments. van Kessel and Russell (1994) reported that pH might be linked to enteric CH_4 emissions (a lower ruminal pH might inhibit CH_4 producing microbes), while van Nevel and Demeyer (1996) reported that the acetic to propionic acid ratio in the rumen is also linked to enteric CH_4 emissions (propionate production inhibits methanogenesis by reducing the availability of metabolic H_2). However, the occurrence of a weak, increasing trend in CH_4 emissions with concentrate supplementation supports the theory regarding ruminal VFA concentrations and pH as individual proxies for enteric CH_4 emissions as indicated by Negussie et al. (2017). In support of this, Aguerre et al. (2011) concluded that CH_4 emissions could not, solely, be predicted from VFA patterns in a study

where the effect of forage-to-concentrate ratio (47 to 68% forage) on CH_4 emissions of dairy cows was evaluated.

It is well documented that there is a strong linear relationship between DMI and enteric CH_4 emissions (Hristov et al., 2013; Knapp et al., 2014; Charmley et al., 2016). However, increasing the OMD or quality of the diet (by feeding grain-based concentrates) may increase the starch:NDF ratio, and because less CH_4 is generated per unit of starch digested than NDF (Moe and Tyrrell, 1979), a reduction in CH_4 emissions (g/d) and intensity (by increased animal production) is expected. Therefore, the slightly higher OMD of the concentrate fed (93%) compared with the pasture offered (87%) was barely sufficient, as supported by the similar NDF intake/body weight between treatments, to increase the diet OMD to a point to maintain daily CH_4 emissions, despite the observed increase in DMI with concentrate supplementation. This occurrence was also evident in the grazing study of Jiao et al. (2014) in which the effect of concentrate level (2, 4, 6, and 8 kg/d) on CH_4 emissions was evaluated.

The observed CH_4 energy (MJ/d) in the current study is within the range of 13.6 to 22.1 as reported by Eckard et al. (2010) for lactating dairy cows, and tended to increase when the pasture-only diet was supplemented with concentrate, regardless of the feeding level. This was probably due to the observed increase in GE intake with increasing concentrate feeding level. The average Y*m* (5.9%) of this study is in agreement with previously reported values of 5.6% (Jiao et al., 2014) and 6.3% (Muñoz et al., 2015). Albeit observing no treatment effect on Y*m*, numerically the values of the current study are similar to that of Tyrrell and Moe (1972), who observed that Y*m* was reduced from 6.4 to 5.1% when the concentrate:forage ratio increased from 0.31 to 0.59 (0 to 0.60 in the current study).

Furthermore, high coefficients of variation (CV) in CH_4 yield could also affect CH_4 emission responses to dietary treatment, and could be accounted for by increasing the statistical power of the SF_6 experiment by increasing animal numbers per treatment. The between-animal CV for CH_4 yield of the few published grazing studies evaluating the effect of concentrate feeding level on CH_4 emissions from dairy cows was not published, therefore making comparisons difficult. Nonetheless, Deighton et al. (2014) reported that previously published between-animal CV ranged from 11 to 24.5%, with their own

between-animal CV reported as low as 6.5% when using their modified SF_6 technique. However, it should be emphasised that CH_4 emissions measured, using the SF_6 technique, during the latter studies, was performed on animals in confinement, and not under grazing conditions that is renowned for the challenges associated with measuring CH_4 emissions and pasture DMI. Even though the between-cow CV in CH_4 yield in the current grazing study was at a high of 31% (21.5% for CH_4 emissions (g/d), and 16.1% for total DMI), CH_4 emission values are in agreement with literature, but may also explain the observed tendencies and lack of response in CH_4 emissions towards an increasing concentrate feeding level, despite the observed increases in milk production and total DMI. In the current study, the implemented strict daily herbage allowance could have caused competitive and aggressive behaviour between cows and some cows may have had variable pasture DMI from day to day. This could be an explanation for the high between-cow CV in CH_4 yield. Therefore, we encourage the use of more than 10 animals to account for high between-animal CV when conducting SF_6 experiments under grazing conditions. Regardless, this study showed that the supplementation of concentrate to a pasture-only diet, increased milk production and total DMI, and linearly decreased CH_4 intensity (g/kg of milk yield).

3.6 CONCLUSIONS

Cows grazed high quality perennial ryegrass pasture under a restricted daily herbage allowance supplemented with three levels of concentrate (0, 4 and 8 kg). The supplementation of concentrate to a pasture-only diet increased animal production, by increasing total DMI, regardless of the concentrate level, and by increasing milk yield and ECM step-wise with increasing concentrate level. Total DMI increased when the pasture-only diet was supplemented with concentrate while CH_4 emissions (g/d) were unchanged. Regardless, CH_4 intensity (g/kg of milk yield) decreased linearly with increasing concentrate feeding level. Results from the rumen study failed to completely support the CH_4 emission results. More research is needed to fully elucidate the role of rumen fermentation parameters as proxies for enteric CH_4 emissions in grazing dairy cows. This study demonstrated that concentrate supplementation to high quality pasture diets has the

potential to effectively reduce CH_4 emissions per unit of milk yield from grazing cows during spring. Results from this study can be used to fine-tune the pasture-based dairy sector of the South African greenhouse gas inventory, and can also be useful for upcoming meta-analysis studies evaluating the effect of diet on enteric CH_4 emissions in improving existing enteric CH_4 prediction equations. Finally, the impact that concentrate supplementation could have on the total carbon footprint, on- and off-farm, as well as the effect on profitability at the farm scale should not be overlooked.

3.7 ACKNOWLEDGEMENTS

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CHAPTER 4

Effect of dietary nitrate on enteric methane emissions, production performance and rumen fermentation of dairy cows grazing kikuyu-dominant pasture during summer

4.1 ABSTRACT

Dietary nitrate supplementation is an effective methane (CH_4) mitigation strategy in total mixed ration based diets fed to ruminants. To date, limited information is available on the effect of dietary nitrate on CH_4 production from grazing dairy cows. Fifty-four multiparous Jersey cows were subjected to a randomised complete block design (blocked according to milk yield, days in milk and parity) to evaluate the effect of three dietary nitrate levels on enteric CH_4 emissions and cow production performance. Additionally, six rumen-cannulated cows in a replicated 3 x 3 Latin square design were used in a rumen study. Dietary treatments consisted of concentrate fed at 5.4 kg of DM/cow per day containing one of three levels of dietary nitrate: 0 g (control), 11 g (low nitrate), and 23 g of nitrate/kg of dry matter (DM; high nitrate). Cows grazed late-summer pasture containing approximately 3 g of nitrate/kg of DM. Concentrates were formulated to be isonitrogenous, by substituting urea, and isoenergetic. Cows were gradually adapted to concentrates over a 3-wk period before the onset of a 57-d experimental period. Enteric CH_4 emissions and total dry matter intake (DMI) from 11 cows per treatment were measured during one 6-d measurement period using the sulphur hexafluoride tracer gas technique. Individual pasture DMI was determined using TiO_2 and indigestible neutral detergent fibre (NDF). Milk yield decreased by approximately 12% when feeding the high nitrate diet compared with the control and low nitrate diets. Although total DMI was unaffected by treatment, concentrate DMI decreased linearly (5.5 to 3.7 kg/d) while pasture DMI increased linearly (9.1 to 11.4 kg/d) with increasing dietary nitrate addition. Methane production (313 to 280 g/d), CH_4 yield (21.8 to 18.7 g/kg of DMI) and CH_4 energy per gross energy intake (6.9 to 5.9%) tended to decrease linearly with increasing dietary nitrate addition. Diurnal ruminal pH of the high nitrate group was greater, for selective periods after concentrate feeding, than the

control and low nitrate groups. Spot sample ruminal pH (6.2 to 6.3) tended to increase while total volatile fatty acid (VFA) concentration (99.9 to 104 m*M*/L) increased quadratically with increasing dietary nitrate addition. Individual VFA concentrations were unaffected by treatment. Rate of NDF disappearance (2.4 to 2.8%/h) after 18 h of ruminal incubation tended to increase quadratically with increasing dietary nitrate addition. Dietary nitrate fed to grazing dairy cows tended to decrease CH_4 emissions while improving the fibrolytic environment of the rumen. However, when feeding high levels of dietary nitrate a decrease in milk yield could be expected due to a decrease in concentrate DMI.

Key words: electron receptor; methane mitigation; pasture-based; SF_6

4.2 INTRODUCTION

Methanogenesis is a natural process in the rumen where enteric methane (CH_4) and water is produced from metabolic hydrogen and carbon dioxide by hydrogenase-expressing bacteria and Archaea in a combined reaction (Knapp et al., 2014). However, CH_4 is a potent greenhouse gas with 28 times the global warming potential of carbon dioxide over a 100 year period (Myhre et al., 2013). With global ruminant numbers increasing annually on average by 26.9 million since 1961 to 2016 (FAO, 2016), the need to abate CH_4 emissions from ruminants is increasing.

Nitrate, an electron receptor, has been labelled as a promising CH_4 mitigation strategy in ruminants (Leng, 2008; Hristov et al., 2013; Lee and Beauchemin, 2014), because the two-step reduction of nitrate to nitrite and, finally, ammonia is energetically more acceptable than methanogenesis (Ungerfeld and Kohn, 2006). Therefore, in recent years interest has increased in the use of dietary nitrate as an efficient CH_4 mitigation strategy (up to 50%) in beef cattle (Newbold et al., 2014; Velazco et al., 2014; Lee et al., 2017) and sheep (Nolan et al., 2010; van Zijderveld et al., 2010; El-Zaiat et al., 2014), but with limited research in lactating dairy cows. To date, only five studies have evaluated the effect of dietary nitrate on CH_4 production from dairy cows, of which all were total mixed ration (TMR)-based and utilised respiration chambers to measure CH_4 emissions (van Zijderveld et al., 2011; Lund et al., 2014; Peterson et al., 2015; Klop et al., 2016; Olijhoek et al., 2016).

Feeding nitrate increases the risk of a potential occurrence of nitrate toxicity, caused by nitrite that is absorbed into the bloodstream and binds with haemoglobin forming methaemoglobin. Methaemoglobin is incapable of carrying oxygen, and high levels of methaemoglobin in blood can occasionally result in asphyxia and death if the animal is not treated immediately (Nolan et al., 2016). Fortunately, critical factors causing nitrate toxicity have been identified and nitrate feeding protocols have been proposed. These include acclimation of animals step-wise to dietary nitrate supplementation for >2 weeks; inclusion of sulphur (nitrite reducing agent) in the nitrate containing diet; and to protect/encapsulate nitrate for slow release (Leng, 2008; van Zijderveld et al., 2010; Lee and Beauchemin, 2014; Nolan et al., 2016).

It is also important to be aware of the basal nitrate content when supplementing dietary nitrate (Leng, 2008). Plants, particularly annual weeds, are prone to accumulate nitrate when the rate of uptake exceeds the rate of nitrate reduction (Maynard et al., 1976; Geuring et al., 1979). Accumulation of nitrate is dependent on plant species, plant growth stage, nitrogen (N) fertiliser application rate (>100 kg of N/ha), light intensity, drought and other plant stress factors causing damage to the plant leaf area (Bolan and Kemp, 2003). The latter emphasises the risk of supplementing dietary nitrate to pasture-based animals, with basal nitrate levels expected to fluctuate at a regular basis, causing sudden peaks in nitrate intake, which can be detrimental to animal production and health. This associated risk of feeding dietary nitrate may, in part, explain the lack of grazing studies supplementing dietary nitrate as a CH_4 mitigation strategy.

However, pasture-based dairy systems improved, unintentionally, to overcome most of the factors possibly responsible for nitrate accumulation in grazing plant species, by: (1) implementing permanent irrigation (overcoming short spells of drought); (2) decreasing N fertilisation rate well below 50 kg of N/ha (overcoming high N input); (3) implementing effective, yet environmentally friendly, weed management (overcoming species that accumulate nitrate); (4) following strict grazing management (avoiding grazing early regrowth, which could be high in nitrate); and (5) planting pasture species, such as legumes, ryegrass (*Lolium* ssp.) and cocksfoot (*Dactylis glomerata*), which are less likely to accumulate nitrate than grain crops (Bolan and Kemp, 2003). Therefore, pasture-based dairy cow research evaluating the effect of dietary nitrate on CH_4 production is justified.

The aim of this study was to determine the effect of dietary nitrate included in the concentrate on CH_4 emissions, production performance and rumen fermentation of Jersey cows grazing kikuyu-dominant pasture during late-summer. We hypothesised that CH_4 production will decrease with increasing dietary nitrate addition.

4.3 MATERIALS AND METHODS

4.3.1 Location and Animal Ethical Clearance

The study was performed in George, Western Cape, South Africa at the Outeniqua Research Farm (33°58´S, 22°25´E), which forms part of the Western Cape Department of Agriculture (Elsenburg, South Africa), and was conducted from February 19 to May 7, 2016. The mean long-term annual precipitation of the experimental area was 732 mm, spread throughout the year, with the mean long-term daily maximum and minimum temperatures varying from 18°C to 25°C, and 7°C to 15°C, respectively. The soil on the 8.55 ha grazing area was a Podzol (Swanepoel et al., 2013). Institutional animal care and use was obtained from the animal ethics committee of the University of Pretoria (project number: EC078-15) before commencement of the study and unnecessary discomfort to the animals was avoided at all times.

4.3.2 Animals, Experimental Design and Treatments

Sixty multiparous Jersey cows (six rumen-cannulated) were selected from the Outeniqua dairy herd with a mean parity of 3.7 (±1.76 SD) and a mean pre-experimental milk yield of 17.5 (±1.21 SD) kg/d, days in milk of 100 (±45.8 SD) d and body weight of 408 (±32.5 SD) kg at the commencement of the study. Intact cows were blocked (18 blocks) according to pre-experimental milk yield, DIM, and parity, in one of three treatment groups on February 5, 2016. The six lactating rumen-cannulated Jersey cows (previously fitted with Bar Diamond #1C rumen cannulae; Bar Diamond Inc, Parma, Idaho, USA) were allocated to the same three groups in a random manner. Cannulated cows formed part of a replicated 3 × 3 Latin square rumen study with 26-d periods (21 d

adaptation and five days data collection). Each 20 cow treatment group was then randomly assigned to one of three concentrate treatments that differed by means of dietary nitrate level: 0, 11 and 23 g/kg of dry matter (DM). The nitrate source was calcium ammonium nitrate [$5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; Yara, Oslo, Norway]. Pelleted concentrate was offered individually to cows at a level of 5.4 kg of DM/cow per day split in two equal portions during milking (0530 h and 1330 h). The nitrate level in the concentrates was based on pre-experimental nitrate content of the grazed pasture (2.13 (±1.36 SD) g of nitrate/kg of DM; n = 10). Concentrates were formulated to be isonitrogenous and isoenergetic (Table 4-1). Limestone ($CaCO_3$) and urea were decremented as the inclusion of the nitrate source increased.

Table 4-1 Ingredient composition (g/kg of DM) of concentrates containing zero (control), low and high levels of nitrate.

	Concentrate treatment		
Item	Control	Low Nitrate	High Nitrate
Ground maize	782	782	782
Soybean oilcake	40	40	40
Wheat bran	50	50	50
Molasses	50	50	50
Monocalcium phosphate	7	7	7
NaCl	5	5	5
Vitamin and trace mineral premix[1]	1	1	1
$MgSO_4$	14	14	15
MgO	2	2	2
$CaCO_3$	30	15	0
Nitrate source[2]	0	24	48
Urea	19	10	0

[1] Containing 4 mg of Cu/kg, 10 mg of Mn/kg, 20 mg of Zn/kg, 0.34 mg of I/kg, 0.2 mg of Co/kg, 0.06 mg of Se/kg, 6×10^6 IU of vitamin A/kg, 1×10^6 IU of vitamin D3/kg, and 8×10^3 IU of vitamin E/kg.
[2] $5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; 750 g NO_3/kg of DM (Yara, Oslo, Norway).

Cows in the control group were allowed three weeks to adapt to the control diet. Whereas cows in the respective nitrate groups were allowed to adapt stepwise to the respective nitrate containing concentrates over a 3-wk period by receiving adaptation concentrates as follow: week one – cows received the first adaptation concentrate containing only one third of the nitrate content of the respective nitrate containing concentrates; week two – cows received two thirds of the nitrate content of the respective nitrate containing concentrates; week three – cows received the respective nitrate containing concentrates. The adaptation concentrates were similar to that of the concentrate treatments, with only the nitrate source, urea, and $CaCO_3$ content changing accordingly.

4.3.3 Pasture and Grazing Management

The experimental grazing area was divided into 15 m × 150 m strips with electric fence and was under permanent sprinkler irrigation. Kikuyu (*Pennisetum clandestinum*) was the dominant (66%) pasture species, followed by perennial ryegrass (17%), other grass (*Lolium multiflorum* and *Paspalum dilatatum*; 14%), white clover (*Trifolium repens*; 6%), and broad-leaf weeds (4%). Pasture strips were top-dressed after each grazing with 42 kg of N/ha using limestone ammonium nitrate (containing 280 g of N/kg). Cows grazed as one group for 24 h per day, except during milking, in a 21-d rotational system with fresh pasture allocated twice daily after milking. Grazing areas were back-fenced. A strict daily herbage allowance was implemented and was constantly adjusted throughout the study to ensure a target post-grazing height of 5.5 cm aboveground level. This was done by taking 100 pasture height readings (pre- and post-grazing) in a zigzag pattern across the grazing area with a rising plate meter (Jenquip folding plate pasture meter; Jenquip, Feilding, NZ). Pasture yield aboveground (pre- and post-grazing) was estimated using the following site-and-season-specific linear regression equation: Pasture yield (kg of DM/ha) = [90 × rising plate meter reading] – 232 (R^2 = 0.84).

4.3.4 Measurements

4.3.4.1 Animal performance

Cows were milked twice daily (0530 h and 1330 h) using a 20-point swing over milking machine with automatic milk yield recording using weigh-all electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Composite morning and afternoon milk samples were taken on one day weekly for milk composition analysis. Milk fat, milk protein, milk lactose and milk urea nitrogen (MUN) content were determined using a Milkoscan FT+ milk analyser (FOSS Analytical, Hillerød, Denmark), and somatic cell count (SCC) was determined using a Fossomatic FC (FOSS Analytical). Energy-corrected milk (ECM) and 4% fat-corrected milk (FCM) was calculated using the equations of Tyrrell and Reid (1965) and Gaines (1928), respectively. Milk parameters from the six rumen-cannulated cows were excluded from the treatment group mean due to the nature of the cross-over design.

Cow body weight (BW) and body condition score (BCS) were recorded prior afternoon milking at the onset and completion of the 8-wk study period. Bodyweight was recorded electronically over two consecutive days with a fixed weighing scale (Tru-Test EziWeigh v. 1.0 scale, 0.5 kg accuracy, Auckland, NZ), while BCS was determined using the 1 to 5 scale scoring system of Wildman et al. (1982).

4.3.4.2 Dry matter intake

Individual pasture DMI was calculated from total faecal output (FO) and forage indigestible neutral detergent fibre (iNDF) using the equation of Cabral et al. (2014): Pasture DMI (kg/d) = [[FO (kg/d) × iNDF faeces (kg/kg)] – iNDF concentrate intake (kg/d)]/iNDF forage (kg/kg). Total FO was calculated using TiO_2 as external marker, from the daily TiO_2 dose and TiO_2 concentration in faeces as described by de Souza et al. (2015). Eleven cows (block 1 to 11) of each treatment group were orally dosed with gelatine capsules (size 10; Torpac Inc., Fairfield, NJ, USA) filled with 3 g of TiO_2/cow twice daily for 10 consecutive d with successive morning and afternoon faecal samples collected from d 6 to d 10 (Pinares-Patiño et al., 2008). Additionally, one cow per treatment was included for background TiO_2 analysis. Faecal samples were immediately

oven dried (65°C, 72 h), pooled within-animal and analysed for TiO_2 concentration by the method of Myers et al. (2004).

For pasture digestibility, daily representative pasture samples were cut (approximately 3 cm aboveground level) during the DMI measurement period on the successive grazing-strip, immediately oven dried (55°C, 72 h), pooled and milled to pass a 1 mm sieve. Pasture, concentrate and faecal iNDF concentrations were determined by incubating the samples *in situ* for 288 h in polyester bags (07-11/5 Sefar Petex cloth, Sefar AG, Heiden, Switzerland), with a sample size to surface area ratio of 12 mg/cm^2, and by determining neutral detergent fibre (NDF) concentration of the residuals after incubation (Krizsan et al., 2015). The NDF concentration of the residual samples were determined by inserting the sealed polyester bags in an Ankom^{200} fibre analyser (Ankom Technology Corp., Fairport, NY, USA) assayed with a heat-stable α-amylase (protein enzyme EC 3.2.1.1; 1,4-α-D-glucan glucanohydrolase) and anhydrous sodium sulphite, and expressed inclusive of residual ash (Robertson and van Soest, 1981).

4.3.4.3 Enteric methane emissions

Enteric CH_4 emissions from individual cows were measured using the sulphur hexafluoride tracer gas (SF_6) technique for grazing dairy cattle as described by O'Neill et al. (2011). This measurement prolonged for six consecutive days (to ensure at least 5 representative gas samples per cow) and was implemented from d 5 to d 10 of the DMI (April 10 to April 15, 2016) measurement period using the same 33 cows as were used to measure DMI by the TiO_2 marker technique. The reason of measuring CH_4 emissions from only 33 of the 54 intact cows was due to a financial constraint. Permeation tubes (P&T Precision Engineering Ltd., Naas, Co. Kildare, Ireland) were filled on-site with 2.9 (±0.19 SD) g of SF_6 gas, during March 2016. Filled permeation tubes were calibrated in a dry incubator (Labcon Incubator Model FS1M8, Johannesburg, South Africa) set at 39.0°C for 27 d weighing (Sartorius BP210S, Sartorius AG, Göttingen, Germany; 0.0001 g accuracy) the tubes in 3-d intervals to produce a 10-point linear regression curve ($R^2 > 0.9996$). The mean release rate of the permeation tubes, 3 d prior dosing, was 5.4 (±0.35 SD) mg of SF_6/d (range: 4.9 to 6.1 mg of SF_6/d). Calibrated permeation tubes were blocked according to release rate and subsequently randomly allocated to experimental cows. Allocated

permeation tubes were individually placed in gelatine capsules (Torpac Inc.) and dosed *per os* on April 3, 2016 (7 d prior to the measurement period).

Cow breath samples were continuously sampled above the nostrils over a 24-h period in evacuated (98 kPa vacuum) polyvinyl chloride (PVC) gas-collection canisters (1700 mL) at a flow rate of approximately 0.54 mL/min. This allowed for the evacuated canisters to fill to 45% over the 24-h sampling period. Crimped stainless-steel capillary tubes (1/16" OD, 0.2" ID; YY-RES-21503; LECO Co., Saint Joseph, MI, USA) were used as inline flow restrictors cut to 50 mm lengths. Canisters were mounted on the back of the cows using the simple back-mounted harness of van Wyngaard et al. (2018a). Sample canisters were reused after flushing residue gas by evacuating to 98 kPa vacuum, filling with ultra-high purity N gas (999.99 g/kg) and evacuating again to 98 kPa vacuum, repeated five times. Canister vacuum was measured with an oil vacuum gauge (SA Gauge (Pty.) Ltd., Durban, South Africa).

Mobile background (ambient) concentrations of SF_6 and CH_4 were sampled throughout the CH_4 measurement period using three additional cows (without permutation tubes) equipped with the same experimental harness, but with the alteration that the flow inlet was located on the back of the animal (pointing down) and not above the nostrils of the animal. Experimental and background cows were kept in one group at all times (grazing and milking). Background gas concentrations in samples collected from all three cows were averaged per day to give a single estimate for all experimental cows.

Undiluted gas samples were extracted from sample canisters using a piston sub-sampler and analysed for SF_6 and CH_4 concentrations using a dual gas chromatograph (Hewlett Packard Model 6890, Palo Alto, CA, USA) with a flame-ionization detector and an electron-capture detector, as described by van Wyngaard et al. (2018b). Methane production (g/d) was calculated using Eq. (2) from the study of Williams et al. (2011).

4.3.4.4 Rumen fermentation

Diurnal ruminal pH patterns were logged over a 72 h period (10 min frequency) using Indwelling TruTrack pH Data Loggers (Model pH-HR mark 4, Intech Instruments Ltd., Christchurch, NZ). Loggers were calibrated with buffer solutions of pH 4 and 9, and verified with buffer solution of pH 7. Logger drift was tested in distilled water for 18 h,

while monitored with a calibrated handheld pH logger (pH340i pH meter/data logger attached with a Sentix 41 pH electrode; WTW, Weilheim, Germany). Ruminal fluid (100 mL) was collected at 8 h intervals (0600, 1400 and 2200 h) from the ventral sac of each cow using a sampling tube attached to a manual vacuum pump. Ruminal pH was immediately measured after sampling with the handheld pH logger (spot sample pH). Subsequently, ruminal fluid were strained through four layers of cheesecloth, subsampled in airtight containers and frozen for subsequent volatile fatty acid (VFA; Filípek and Dvořák, 2009) and NH_3-N (Broderick and Kang, 1980) analysis. Dry matter and NDF *in sacco* disappearance (after 6, 18 and 30 h incubation) of the grazed pasture were determined using the nylon bag technique of Cruywagen (2006). Bag residuals were analysed for DM content (AOAC, 2000; method 934.01), NDF content (as described previously in section 4.3.4.2), and acid detergent fibre content (Goering and van Soest, 1970). Rate of NDF disappearance (NDF k_d) was calculated according to van Amburgh et al. (2003).

4.3.5 Feed Sampling and Analysis

Representative pasture and concentrate samples were collected on a weekly basis, dried at 55°C for 72 h (initial DM), ground to pass through a 1 mm sieve (SMC hammer mill) and stored at −18°C pending analyses. One pasture sample consisted of 6 pooled pasture samples cut approximately 3 cm aboveground level from the successive grazing-strip. Homogenised samples were analysed for DM, ash, crude protein (CP; N content determined using a LECO Trumac^{TM} N Determinator, LECO Corporation, Saint Joseph, MI, USA) and ether extract, according to procedures of AOAC (2000; methods 934.01, 942.05, 968.06 and 920.39, respectively). The NDF content was determined as described previously in section 4.3.4.2, while acid detergent fibre was determined according to Goering and van Soest (1970) using the Ankom^{200} fibre analyser. Samples were also analysed for *in vitro* organic matter digestibility (Tilley and Terry, 1963; using rumen fluid from a rumen-cannulated SA Mutton Merino ram fed good-quality lucerne hay), and gross energy (GE; MC–1000 modular calorimeter, Energy Instrumentation, Sandton, South Africa; operator's manual), while metabolisable energy (ME) was calculated using the

equations of MAFF (1984). Mineral composition and nitrate content was determined according to procedures of AgriLASA (1998, method 6.1.1; and 2004, respectively).

4.3.6 Statistical Analysis

Individual production variables measured daily (milk yield, DMI, and CH_4 parameters) and weekly (milk composition parameters) were averaged within-cow representative of the 8-wk study period and the CH_4 measurement period. A 91% successful collection rate was achieved from the 196 samples of gas intended to be collected. The failed sample collections were due to blockages in the capillary flow restrictor, and broken sampling lines during the 24 h collection periods. The modified Z-score was used to identify outlying CH_4 data. Data associated with 'modified Z-scores' of >3.5 (absolute value) were labelled as outliers (Berndt et al., 2014).

Milk production and cow body condition parameters (18 blocks) over the course of the 8-wk study period, and DMI parameters and CH_4 emissions (11 blocks) over the course of the CH_4 measurement period were analysed as a randomised complete block design with ANOVA to test for differences between treatment effects. Residuals were acceptably normal with homogeneous treatment variances, except for SCC, which were then log (base 10) transformed. Covariate analysis was done using pre-experimental milk yield, DIM and parity as covariates but no significant relationships were found; hence, excluded from the statistical analysis.

Rumen variables (ruminal fluid pH, fermentation end-products, and kinetic parameters of pasture DM and NDF) were analysed as a replicated 3 × 3 Latin square testing for differences between treatment effects.

Treatment means were compared using Tukey's least significant difference test at the 5% level of significance (Snedecor and Cochran, 1980). Data were analysed using the statistical program GenStat (Payne et al., 2014).

4.4 RESULTS

4.4.1 Feed Chemical Composition and Pasture Management

The chemical composition of the dairy concentrate and pasture offered averaged across the 8-wk study period is presented in Table 4-2. The respective concentrate treatments contained on average 0, 11 and 23 g of nitrate/kg of DM. Grazed pasture contained 3.2 g of nitrate/kg of DM averaged over the 8-wk study period with a range of 1.3 to 4.4 g of nitrate/kg of DM (results not shown).

Table 4-2 Chemical composition (g/kg of DM, or as stated) of concentrates containing zero (control), low and high levels of nitrate and of the pasture offered averaged (±SD) over the 8-wk study period.

Item[1]	Concentrate treatment (n = 4)			Pasture[3] (n = 18)
	Control	Low Nitrate	High Nitrate	
Initial DM (g/kg)	909 ± 4.9	902 ± 3.1	904 ± 0.2	174 ± 21.5
DM composition (g/kg)				
CP	144 ± 0.1	146 ± 0.1	140 ± 0.3	192 ± 19.9
Nitrate[2]	0	11	23	3.2 ± 1.07
EE	29 ± 2.8	21 ± 0.9	21 ± 0.1	25 ± 3.1
NDF	146 ± 10.1	119 ± 10.3	120 ± 5.8	584 ± 35.9
ADF	27 ± 1.4	29 ± 1.4	30 ± 2.1	293 ± 21.4
Ash	70 ± 2.3	68 ± 0.3	83 ± 5.9	107 ± 12.1
GE (MJ/kg of DM)	17.1 ± 0.07	17.1 ± 0.02	16.6 ± 0.08	17.8 ± 0.28
ME (MJ/kg of DM)	14.0 ± 0.25	14.2 ± 0.17	13.8 ± 0.13	9.38 ± 1.37
Mineral composition (g/kg)				
Ca	14 ± 0.4	14 ± 0.1	16.0 ± 0.92	4.3 ± 0.77
P	5.5 ± 0.15	5.7 ± 0.06	5.7 ± 0.28	4.4 ± 0.75
Mg	4.5 ± 0.15	4.8 ± 0.15	5.9 ± 0.35	5.1 ± 0.81
K	8.2 ± 0.13	8.2 ± 0.06	8.0 ± 0.07	38 ± 6.1
Cu (mg/kg of DM)	32 ± 7.9	26 ± 3.5	26.3 ± 0.64	9.0 ± 1.14
Fe (mg/kg of DM)	186 ± 2.8	161 ± 3.5	171 ± 22.3	198 ± 66.0

[1] DM–dry matter; CP–crude protein; EE–ether extract; NDF–neutral detergent fibre; ADF–acid detergent fibre; GE–gross energy; ME–metabolisable energy.
[2] Sample represents four pooled concentrate samples.
[3] Pasture – kikuyu (*Pennisetum clandestinum*) dominant.

The pre- and post-grazing measurements of the offered pasture between the 8-wk study period and the CH_4 measurement period are presented in Table 4-3. Cows were offered pasture at 11.5 kg of DM/cow per day, 3 cm aboveground level, and the average pasture yield was 2.3 t of DM/ha. According to the pre- and post-grazing measurements, cows consumed daily approximately 67% and 82% of the pasture offered during the 8-wk study period and CH_4 measurement period, respectively.

Table 4-3 Pre- and post-grazing measurements of the kikuyu-dominant pasture averaged (±SD) across the 8-wk study period and the methane measurement period.

Item[1]	8-wk study period (n = 60)	Methane measurement period (n = 11)
Pasture height (cm)		
Pre-grazing	13.9 ± 2.27	12.9 ± 2.33
Post-grazing	6.10 ± 0.628	6.12 ± 0.516
Pasture yield (kg of DM/ha)[2]		
Pre-grazing	2252 ± 408.3	2095 ± 419.4
Post-grazing	868 ± 113.2	871 ± 93.0
DHA (kg of DM/cow per day)	11.5 ± 1.78	9.74 ± 2.127
Daily grazed area (m^2/cow)	58.8 ± 14.71	67.5 ± 14.41
Pasture removed (kg of DM/cow per day)	7.69 ± 1.820	8.03 ± 3.047

[1] DM–dry matter; DHA–daily herbage allowance.
[2] Pasture yield (kg of DM/ha) = (90 × rising plate meter reading) – 232 (R^2 = 0.84); estimated 3 cm aboveground level using a rising plate meter.

4.4.2 Milk Production, Milk Composition and Cow Condition

Milk yield decreased linearly and quadratically ($P<0.05$) with increasing dietary nitrate addition, while FCM and ECM decreased linearly ($P<0.001$) with ECM showing a tendency to decrease quadratically ($P=0.065$) with increasing dietary nitrate addition (Table 4-4). Milk yield, FCM and ECM were lowest ($P<0.01$) for the high nitrate treatment compared with the control and low nitrate treatments. Correspondingly, cows on the high nitrate diet had a smaller ($P<0.001$) milk fat yield and protein yield in comparison with the other treatments. Cows on the control diet had a similar milk lactose yield than cows on the nitrate containing diets, while cows on the high nitrate diet had a smaller ($P=0.012$) milk lactose yield than cows on the low nitrate diet. Milk fat and milk protein yield decreased

linearly (P<0.01) with increasing dietary nitrate addition. Additionally, milk protein and milk lactose yield decreased quadratically (P<0.05) with milk lactose yield showing a tendency to decrease linearly (P=0.052) with increasing dietary nitrate addition. Milk fat content decreased quadratically (P=0.041), while milk protein to fat content ratio increased quadratically (P=0.001) with increasing dietary nitrate addition. Cows on the low nitrate diet, compared with cows on the control and high nitrate diet, had a greater (P=0.005) milk protein to fat ratio. Milk lactose content tended to increase linearly and quadratically (P<0.10) with increasing dietary nitrate addition. Body condition parameters were unchanged by dietary nitrate supplementation.

Table 4-4 Milk production and cow condition of early lactation Jersey cows grazing kikuyu-dominant pasture in late-summer fed concentrates containing zero (control), low and high levels of nitrate averaged across the 8-wk study period.

Number of cows	18	18	18				
Item[1]	Concentrate treatment[2]			SEM[3]	P-value[4]		
	Control	Low Nitrate	High Nitrate		Con	Lin	Quad
Milk yield (kg/d)	13.5[a]	13.8[a]	12.0[b]	0.38	0.005	0.009	0.035
FCM yield (kg/d)	16.9[a]	16.5[a]	14.9[b]	0.37	<0.001	<0.001	0.21
ECM yield (kg/d)	16.4[a]	16.2[a]	14.5[b]	0.35	<0.001	<0.001	0.065
Milk fat (g/kg)	57.4	53.8	56.7	1.27	0.11	0.69	0.041
Milk protein (g/kg)	36.5	37.3	36.5	0.54	0.42	0.98	0.19
Milk protein to fat ratio	0.64[b]	0.70[a]	0.65[b]	0.013	0.005	0.59	0.001
Milk lactose (g/kg)	43.6	45.0	44.6	0.39	0.055	0.096	0.075
Milk solids (g/kg)	138	136	138	1.4	0.68	0.91	0.39
MUN (mg/dL)	11.5	11.8	11.9	0.31	0.75	0.47	0.83
Log_{10} SCC	1.92	2.12	2.11	0.084	0.18	0.11	0.33
Milk fat yield (kg/d)	0.77[a]	0.73[a]	0.67[b]	0.017	<0.001	<0.001	0.61
Milk protein yield (kg/d)	0.49[a]	0.51[a]	0.43[b]	0.011	<0.001	0.001	0.002
Milk lactose yield (kg/d)	0.59[ab]	0.62[a]	0.53[b]	0.019	0.012	0.052	0.020
Initial BW (kg)	412	401	409	6.8	0.55	0.75	0.30
Initial BCS (scale 1 to 5)	2.15	2.14	2.16	0.032	0.89	0.87	0.65
BW change (kg)	-24.8	-21.1	-28.1	3.20	0.32	0.48	0.18

[a,b,c] Row means with different superscripts differ significantly at P<0.05.
[1] FCM–4% fat corrected milk; ECM–energy corrected milk; Milk solids = milk fat + milk protein + milk lactose; MUN–milk urea nitrogen; SCC–somatic cell count; BW–body weight; BCS–body condition score.
[2] Concentrate fed at 5.4 kg (dry matter basis)/cow per day split in two equal portions during milking, containing either 0 (control), 11 (low nitrate) or 23 g of nitrate/kg of dry matter.
[3] SEM–standard error of mean.
[4] Con–contrast; Lin–linear; Quad-quadratic.

Table 4-5 Individual faecal output, body weight, dry matter intake and enteric methane emissions of early lactation Jersey cows grazing kikuyu-dominant pasture in late-summer fed concentrates containing zero (control), low and high levels of nitrate averaged across the methane measurement period.

Number of cows	11	11	11				
Item[1]	Concentrate treatment[2]			SEM[3]	P-value[4]		
	Control	Low Nitrate	High Nitrate		Con	Lin	Quad
FO (kg of DM/d)	3.01	2.93	2.88	0.136	0.79	0.68	0.59
BW (kg)	407	385	383	7.5	0.061	0.034	0.27
Intake							
Pasture DMI (kg/d)	9.14[b]	9.67[b]	11.4[a]	0.450	0.006	0.002	0.30
Concentrate DMI (kg/d)	5.45[a]	5.41[a]	3.66[b]	0.074	<0.001	<0.001	<0.001
Total DMI (kg/d)	14.6	15.1	15.0	0.45	0.72	0.53	0.63
NDF intake as % of BW	1.51[b]	1.65[ab]	1.86[a]	0.076	0.014	0.004	0.69
DMI as % of BW	3.59	3.94	3.94	0.138	0.14	0.085	0.32
GEI (MJ/d)	256	265	265	7.9	0.67	0.44	0.67
MEI (MJ/d)	162	167	158	4.2	0.36	0.47	0.22
CH_4 emissions							
CH_4 production (g/d)	313	300	280	11.4	0.15	0.057	0.83
CH_4/DMI (g/kg)	21.8	20.1	18.7	1.13	0.19	0.070	0.92
CH_4/milk yield (g/kg)	24.2	22.7	25.3	1.43	0.45	0.59	0.26
CH_4/ECM (g/kg)	19.7	19.1	20.8	0.91	0.41	0.39	0.30
CH_4 energy (MJ/d)	17.3	16.5	15.5	0.63	0.15	0.055	0.84
Y*m* (%)	6.85	6.32	5.86	0.358	0.17	0.064	0.94

[a,b,c] Row means with different superscripts differ significantly at P<0.05.
[1] FO–faecal output; DM–dry matter; BW–body weight; DMI–dry matter intake; NDF–neutral detergent fibre; GEI–gross energy intake; MEI–metabolisable energy intake; CH_4–methane; ECM–energy-corrected milk; CH_4 energy = (55.22 MJ*CH_4 g/d)/1000; Y*m*–methane energy per gross energy intake.
[2] Concentrate fed at 5.4 kg (dry matter basis)/cow per day split in two equal portions during milking, containing either 0 (control), 11 (low nitrate) or 23 g of nitrate/kg of dry matter.
[3] SEM–standard error of mean.
[4] Con–contrast; Lin–linear; Quad-quadratic.

4.4.3 Dry Matter Intake and Enteric Methane Emissions

Body weight of cows decreased linearly (P=0.034), while pasture DMI increased linearly (P=0.002) with increasing dietary nitrate addition (Table 4-5). The high nitrate diet fed to cows resulted in a greater (P=0.006) pasture DMI compared with cows fed either the control or low nitrate diets. Conversely, cows fed the high nitrate diet had a lower (P<0.001) concentrate DMI compared with cows on the other two treatment diets. Concentrate DMI decreased linearly and quadratically (P<0.001) with increasing dietary nitrate addition. Total DMI was, however, unaffected by treatment. Individual NDF intake as % of BW increased linearly (P=0.004) with increasing dietary nitrate addition, and was greater (P=0.014) for cows on the high nitrate diet than cows on the control diet, but similar to cows on the low nitrate diet. Total DMI as % of BW tended to increase linearly (P=0.085) with increasing dietary nitrate addition.

Methane production (g/d), CH_4 yield (g/kg of DMI), CH_4 energy and Y*m* tended to decrease linearly (P<0.10) with increasing dietary nitrate addition. Methane intensity (g/kg of milk yield, and kg of ECM) was unaffected by treatment.

4.4.4 Rumen Fermentation

Diurnal ruminal fluid pH of cows in the high nitrate group was higher (P<0.05) than the other groups following 1 h after morning feeding of concentrate, and remained higher (P<0.05) for five consecutive hours before stabilising (Figure 4-1). Subsequently, after afternoon feeding of concentrate, diurnal ruminal pH of cows in the high nitrate group was greater (P<0.05) than the other groups for 11 consecutive hours before stabilising. Thereafter, intermittent increases (P<0.05) in diurnal ruminal pH was evident for the high nitrate treatment group in comparison with the other treatment groups. The overall mean diurnal ruminal pH over 72 h was, however, unchanged by nitrate supplementation, regardless of the inclusion level (Table 4-6). Spot sample pH taken concurrently with rumen fluid collection tended to increase linearly (P=0.082) with increasing dietary nitrate addition. Furthermore, hours spent below diurnal ruminal pH of 6.6 and 6.4 decreased linearly (P<0.05) with increasing dietary nitrate addition.

Total VFA concentration increased quadratically (P=0.008) with increasing dietary nitrate addition, and was greater (P=0.019) for cows on the low nitrate diet compared with cows on the control diet, but similar to cows on the high nitrate diet. Individual VFA concentrations, and *in sacco* DM and NDF disappearances were unaffected by treatment. However, NDF k_d after 18 h of ruminal incubation increased quadratically (P=0.047) and tended to increase linearly (P=0.092) with increasing dietary nitrate addition, being greater (P=0.051) for the high nitrate group in comparison with the low nitrate group, but similar to the control group.

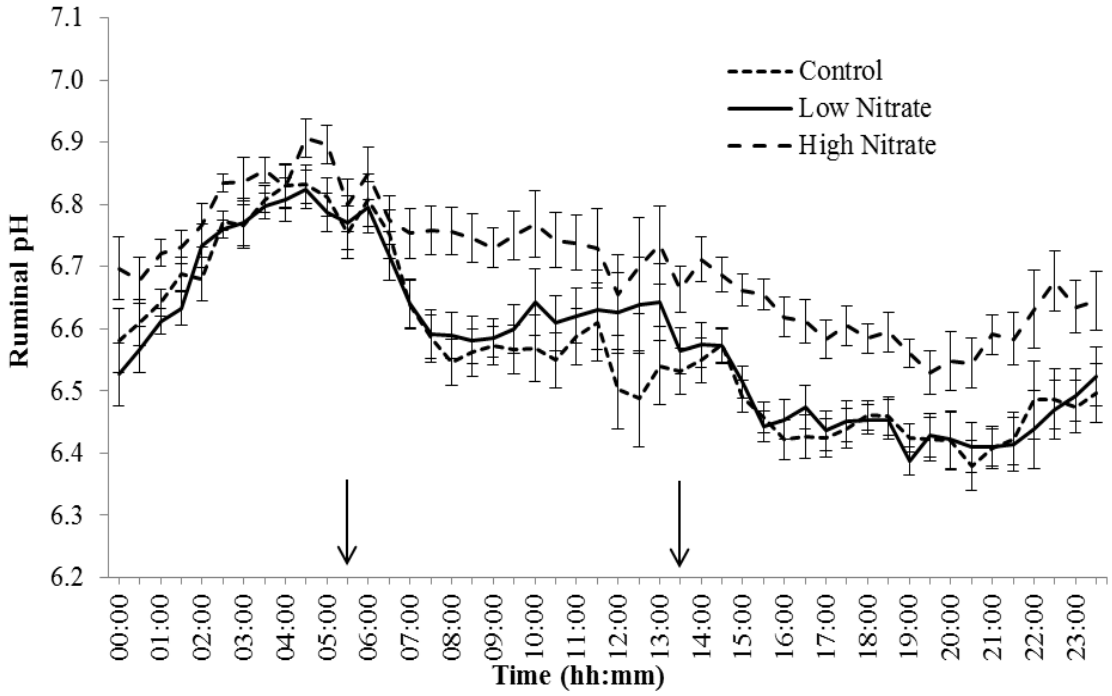

Figure 4-1 Diurnal ruminal pH of early lactation Jersey cows (rumen-cannulated) grazing kikuyu-dominant pasture in late-summer fed concentrates containing zero, low and high levels of nitrate (n = 6). Concentrate fed at 5.4 kg (dry matter basis)/cow per day split in two equal portions during milking, containing either 0 (control), 11 (low nitrate) or 23 g of nitrate/kg of dry matter. Error bars indicate standard error of mean and arrows indicate when concentrate was fed.

Table 4-6 Ruminal fluid pH, concentrations of NH_3-N, total volatile fatty acid and percentages of individual volatile fatty acids as well as kinetic parameters of pasture dry matter and neutral detergent fibre in early lactation Jersey cows (rumen-cannulated) grazing kikuyu-dominant pasture in late-summer fed concentrates containing zero (control), low and high levels of nitrate.

Number of cows	6	6	6				
Item[1]	Concentrate treatment[2]			SEM[3]	P-value[4]		
	Control	Low Nitrate	High Nitrate		Con	Lin	Quad
Diurnal pH (over 72 h)	6.53	6.59	6.74	0.086	0.28	0.13	0.70
Spot sample pH	6.20	6.29	6.31	0.038	0.16	0.082	0.43
Time below (h)							
pH 6.0	0.75	0.58	0.00	0.233	0.094	0.23	0.68
pH 6.2	1.83	1.92	0.50	1.045	0.55	0.28	0.47
pH 6.4	4.83	5.25	2.33	2.489	0.66	0.047	0.10
pH 6.6	12.4	12.6	5.92	4.063	0.42	0.041	0.17
NH_3-N (mg/dL)	15.7	17.1	16.5	2.16	0.90	0.81	0.71
Total VFA (m*M*/L)	99.3[b]	117[a]	104[ab]	3.25	0.019	0.31	0.008
Acetic (m*M* %)	63.7	64.2	64.1	0.73	0.87	0.71	0.74
Propionic (m*M* %)	19.4	18.1	18.4	0.63	0.40	0.31	0.38
Butyric (m*M* %)	13.5	14.3	14.1	0.42	0.39	0.33	0.34
Isobutyric (m*M* %)	0.92	0.92	0.96	0.050	0.83	0.62	0.75
Valeric (m*M* %)	1.14	1.11	1.10	0.057	0.85	0.59	0.93
Isovaleric (m*M* %)	1.06	0.98	1.05	0.079	0.73	0.98	0.44
Caproic (m*M* %)	0.39	0.37	0.37	0.018	0.62	0.43	0.59
DM disappearance (coeff.)							
6 h	0.21	0.22	0.22	0.008	0.76	0.49	0.86
18 h	0.37	0.36	0.41	0.015	0.19	0.17	0.20
30 h	0.51	0.53	0.53	0.017	0.60	0.37	0.69
NDF disappearance (coeff.)							
6 h	0.03	0.04	0.03	0.013	0.93	0.92	0.73
18 h	0.23	0.22	0.26	0.018	0.23	0.22	0.22
30 h	0.42	0.45	0.44	0.022	0.66	0.53	0.52
NDF k_d (per hour)							
6 h	0.007	0.008	0.007	0.0027	0.92	0.90	0.71
18 h	0.024[ab]	0.021[b]	0.028[a]	0.0015	0.051	0.092	0.047
30 h	0.028	0.031	0.030	0.0024	0.72	0.61	0.54
Mean	0.019	0.020	0.022	0.0019	0.69	0.42	0.84

[a,b,c] Row means with different superscripts differ significantly at P<0.05.

[1] NH_3-N–ammonia nitrogen; VFA–volatile fatty acid; DM–dry matter; NDF–neutral detergent fibre; NDF k_d–rate of neutral detergent fibre disappearance.

[2] Concentrate fed at 5.4 kg (dry matter basis)/cow per day split in two equal portions during milking, containing either 0 (control), 11 (low nitrate) or 23 g of nitrate/kg of dry matter.

[3] SEM–standard error of mean.

[4] Con–contrast; Lin–linear; Quad-quadratic.

4.5 DISCUSSION

It is believed that dietary nitrate is the only feed additive that can persistently mitigate CH_4 production without adverse effects on milk production in dairy cattle, but it comes with an animal toxicity concern (Knapp et al., 2014). However, previous TMR-based dairy studies demonstrated the efficacy of nitrate to decrease CH_4 production with only minor increases in blood methaemoglobin (indicator for nitrate poisoning) well below near-toxic thresholds (van Zijderveld et al., 2011; Klop et al., 2016; Olijhoek et al., 2016). This research is the first of its kind to evaluate the effect of dietary nitrate on CH_4 emissions from grazing dairy cows.

Average CH_4 emission results of this study are in line with previous grazing studies (Jiao et al., 2014; Muñoz et al., 2015). Nitrate intakes of the current treatment groups were 2, 6, and 8 g of nitrate/kg of DM, or 0.07, 0.24, and 0.31 g of nitrate/kg of BW for the control, low nitrate and high nitrate groups, respectively, given the measured pasture and concentrate DMI of the current study. Theoretically, by implementing the CH_4 yield prediction equation of Lee and Beauchemin (2014), CH_4 yield (g/kg of DMI) = –8.3 × nitrate (g/kg of BW) + 15.2, it is predicted that the low and high nitrate treatment will reduce CH_4 yield by 10% and 15%, respectively, in comparison to the control group. In agreement, in the current study, the low and high nitrate treatments tended to reduce CH_4 yield by 8% and 15%, respectively. This indicates that the nitrate treatment effect on CH_4 emissions in this study is in agreement with previous findings.

The observed milk production and rumen parameters in this study were mostly within range of values reported in a review study evaluating the effects of supplementation on production parameters of grazing dairy cows (Bargo et al., 2003). Milk urea nitrogen and ruminal NH_3-N were within acceptable ranges for pasture-based dairy cows (Bargo et al., 2003), indicating that dietary N was not deficiently or in excess. The lack of a response in milk composition to the addition of dietary nitrate in the current study was also observed by previous nitrate studies on dairy cows (van Zijderveld et al., 2011; Olijhoek et al., 2016). However, van Zijderveld et al. (2011) reported a decrease in milk protein content when nitrate was fed that was mainly a consequence of dilution and not a nitrate treatment effect. Both the latter studies reported decreases in CH_4 production but with simultaneous

increases in enteric hydrogen production. This indicates that feed energy saved due to the decrease in CH_4 production was not converted to milk production but rather, in part, utilised for enteric hydrogen production, because hydrogen emissions constitute a loss of ingested energy (Lee and Beauchemin, 2014). Although enteric hydrogen was not measured in the current study, prolonged periods of increased ruminal pH soon after feeding of the high nitrate containing concentrate indicates that hydrogen peaked during these periods in the rumen. Peaks in hydrogen were also observed by Olijhoek et al. (2016) soon after feeding nitrate to dairy cows.

Stoichiometrically, when 100 g of nitrate is fully reduced to ammonia in the rumen CH_4 emissions is reduced by 25.8 g (Lee and Beauchemin, 2014). Assuming that pasture and concentrate DMI where unchanged in the current study and that the CH_4 decreases were statically significant, the calculated stoichiometric CH_4 reducing efficiency of the nitrate levels fed in the low and high nitrate diets (above the nitrate level of the pasture) would be 83% and 98%, respectively. However, the reduced concentrate DMI of the high nitrate group resulted in a surprising 142% CH_4 reducing efficiency. Previous nitrate studies using dairy cows reported average CH_4 reducing efficiencies of 78% to 86% (Lund et al.., 2014; Klop et al., 2016; Olijhoek et al., 2016), whereas van Zijderveld et al. (2011) reported a lower value of 59%. However, Olijhoek et al. (2016) reported that there were instances when CH_4 reducing efficiencies of individual cows were above 100%, with a maximum observed CH_4 reducing efficiency of 142%, the same as reported in our study. This greater efficiency may indicate that the CH_4 reducing effect of nitrate was not only related to its electron capturing ability, but feasibly to a toxic effect exerting antimicrobial effects that can impede rumen fermentation (Kluber and Conrad, 1998), or other factors that still need to be established.

Based on the ruminal metrics reported in Table 4-6, we can conclude that dietary nitrate addition in this study did not adversely affect the rumen fermentation results, indicating that nitrate toxicity was likely not present during this study. Correspondingly, previous *in vivo* (Olijhoek et al., 2016) and *in vitro* (Lund et al., 2014) studies using dairy cows also concluded that the addition of dietary nitrate did not impede rumen fermentation. The quadratic increase in total VFA concentration observed in the current study could be ascribed to the possible increase in enteric hydrogen. In agreement, Olijhoek et al. (2016)

observed a tendency in total VFA concentrations to increase with the addition of dietary nitrate. The authors ascribed the tendency to the observed increase in hydrogen.

Although individual total DMI was unaffected by nitrate supplementation in the current study, it was observed that the high nitrate diet decreased concentrate DMI and milk yield, while pasture DMI increased correspondingly. Both van Zijderveld et al. (2011) and Olijhoek et al. (2016) reported that total DMI and milk yield were unchanged by addition of nitrate (21, and 6 to 23 g of nitrate/kg of DM, respectively) in TMR diets fed to dairy cows that were gradually adapted to nitrate. On the contrary, Lund et al. (2014), Peterson et al. (2015), and Klop et al., (2016) reported that total DMI decreased by 11%, 27%, and 5%, respectively, when nitrate was fed at 20, 21, and 21 g/kg of DM, respectively. However, it should be noted that cows from the study of Lund et al. (2014) were not adapted to nitrate, whereas it is unclear whether cows from the study of Peterson et al. (2015) were adapted to nitrate or not. Cows in the study of Klop et al. (2016) were, however, gradually adapted. Furthermore, Hegarty et al. (2013) demonstrated that by not gradually adapting beef cattle to a nitrate-based diet (9.5 g of nitrate/kg of DM), DMI, average daily gain and carcass weight were lower compared with cattle fed a urea-based diet. These authors reported that a lower DMI imposed by dietary nitrate addition signifies one of the symptoms related to sub-acute nitrate toxicity. Therefore, it is clear that animals need to be gradually adapted to nitrate to avoid negative effects on DMI and animal production. This is supported by Lee and Beauchemin (2014) who reported that dietary adaptation is essential to sustain high levels of DMI and animal production when feeding nitrate especially at levels greater than 25 g of nitrate/kg of DM. Cows in the current study were gradually adapted to nitrate diets. Although blood methaemoglobin was not measured during this study, it can be said that nitrate toxicity was unlikely to be the cause of the observed reduction in concentrate DMI. Another explanation for the decrease in DMI might be due to the bitter taste of nitrate resulting in a reduced palatability of the nitrate containing feed (Bruning-Fann and Kaneene, 1993). Even in encapsulated form, the addition of nitrate to TMR diets resulted in sorting against nitrate (Lee et al., 2017). Thus, the observed decrease in concentrate DMI in the current study without affecting total DMI is, in part, explained by the organoleptic issue of nitrate. Possible flavourants for nitrate containing diets, especially in concentrate form, deserve further study.

Cows on the high nitrate diet increased their pasture DMI in an attempt to compensate for the decrease in concentrate DMI. Pasture substitution was reversed. However, unsupplemented pasture, irrespective of digestibility, is unable to supply sufficient energy to meet the requirements of high producing dairy cows (Bargo et al., 2003), because pasture DMI in dairy cows is limited by several factors such as rumen fill (Boudon et al., 2009). Therefore, the observed increase in pasture DMI in the current study was inadequate to supply the energy lost by the partial refusal of concentrate. Although ME intake was unaffected by nitrate addition in the current study, a numerical difference in ME intake of 4 MJ/cow per d was evident between the control and high nitrate groups. Given the cow production parameters in the current study a ME margin of 4 MJ/cow per d could result in approximately 1 kg difference in milk yield (NRC, 2001), therefore partially explaining the observed decrease in milk yield for cows on the high nitrate diet.

Pasture composition parameters in the current study are comparable with those reported in a previous South African pasture-based study for high quality, N-fertilised kikuyu-dominant pasture during late-summer (van der Colf et al., 2015). Although non-protein nitrogen (NPN) content was not determined, it was previously reported that N-fertilised kikuyu has an inherently higher NPN content than temperate species such as ryegrass (Reeves et al., 1996). Further research on the use of dietary nitrate as CH_4 mitigation strategy for dairy cows grazing pasture species with inherent lower NPN fractions compared with kikuyu is warranted.

Care should be taken when feeding nitrate because it can result in increased N_2O emissions from both the animal and manure. Nitrous oxide is also a potent greenhouse gas (Myhre et al., 2013). The simultaneous release of N_2O along with CH_4 by cows fed dietary nitrate may partly offset the CH_4 mitigation potential of dietary nitrate by as much as 1.4 – 3.2%, 5.7 – 76% (the latter might be an outlier), and 10.1 – 14.8% when fed at levels of 5, 14, and 21 g of nitrate/kg of DM (Peterson et al., 2015). However, the range of the latter study consists of measurements from only two cows from different measurement periods and should, therefore, be interpreted with caution.

4.6 CONCLUSIONS

This study demonstrated that feeding concentrate containing 23 g of nitrate/kg of DM (total nitrate intake of 8 g of nitrate/kg of DM) to grazing dairy cows may result in partial concentrate refusal; hence, decreasing milk yield. It was believed that the partial refusal of concentrate was manifested by the organoleptic properties of the high nitrate concentrate and not as a result of nitrate toxicity, because total DMI was unaffected by treatment. Dietary nitrate fed to grazing dairy cows tended to decrease CH_4 emissions while improving the fibrolytic environment of the rumen. Therefore, dietary nitrate could potentially be a CH_4 mitigation strategy for pasture-based systems; hence justifying further research on different pasture species as affected by season.

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CHAPTER 5

Effect of dietary nitrate on enteric methane emissions, production performance and rumen fermentation of dairy cows grazing ryegrass pasture during spring

5.1 ABSTRACT

Limited studies investigated the effect of dietary nitrate addition as methane (CH_4) mitigation strategy for dairy cows grazing pasture. This study aimed to investigate the effect of dietary nitrate addition on daily enteric CH_4 emissions, production performance and rumen fermentation of multiparous Jersey cows grazing perennial ryegrass pasture (containing approximately 7.3 g of nitrate/kg of dry matter (DM)). Thirty-two intact and eight rumen-cannulated multiparous Jersey cows were subjected to a replicated 2 × 2 Latin square design with 16 intact cow replicates and four rumen-cannulated cow replicates supplemented with one of two concentrates containing either urea (urea treatment), or urea and nitrate (nitrate treatment) as non-protein nitrogen source (NPN; containing 0.3 and 15.2 g of nitrate/kg of DM, respectively). Concentrates were formulated to be isonitrogenous and isoenergetic, and was fed at 5.4 kg of DM/cow per d along with a strict daily herbage allowance of 14 kg of DM/cow. Cows were gradually adapted to concentrates over a 3-wk period. Daily enteric CH_4 emissions of 28 cows were measured with the sulphur hexafluoride tracer gas technique for six consecutive days during each experimental period with parallel total DM intake (DMI) estimates. Pasture DMI was calculated from faecal output and pasture digestibility using TiO_2 and indigestible neutral detergent, respectively. Total DMI (18.1 and 17.8 kg/d), milk yield (19.0 and 18.7 kg/cow per d) and daily CH_4 emissions (400 and 405 g/d) were unaffected by dietary treatment for the urea and nitrate group, respectively. Total milk solids content (135 *vs.* 133 g/kg), milk fat content (50.8 *vs.* 48.5 g/kg), milk lactose content (47.3 *vs.* 46.7 g/kg) and milk urea nitrogen concentration (MUN; 12.6 *vs.* 11.6 mg/dL) were higher for the nitrate group. Rumen fermentation parameters such as volatile fatty acid profile, ammonium nitrogen, and DM and fibre disappearance were unaffected by treatment. Minor effects on ruminal

pH were observed with an increasing tendency towards the nitrate group. Although dietary nitrate supplementation is not an effective CH_4 mitigation strategy for dairy cows grazing perennial ryegrass, increases in milk fat and lactose content may be expected.

Key words: CH_4 measurement; perennial ryegrass; methane mitigation; urea; SF_6

5.2 INTRODUCTION

Globally, ruminants represent 39% of the livestock sector's greenhouse gas emissions in the form of enteric methane (CH_4; Gerber et al., 2013). Methane is a destructive greenhouse gas with 28 times the global warming potential than that of carbon dioxide over a 100 y period (Myhre et al., 2013), and connotes a loss of gross energy intake. Strategies that can potentially reduce enteric CH_4 could, therefore, be of high significance in the cause to mitigate greenhouse gasses on a global scale.

Enteric CH_4 is naturally produced mainly in the rumen by a process called methanogenesis which is performed by methanogenic archaea that primarily utilises H_2 and CO2 as substrates for enteric CH_4 production (Morgavi et al., 2010). Therefore, the introduction of an alternative hydrogen sink in the rumen could limit CH_4 emissions from ruminants. Among existing enteric CH_4 reducing compounds, nitrate as alternative hydrogen sink has shown the most persistent CH_4 reducing capabilities with least or no adverse effects on animal production, but has variable efficacy and could cause nitrate toxicity in animals (Hristov et al., 2013; Knapp et al., 2014; Yang et al., 2016). In recent dietary nitrate studies, the occurrence of nitrate toxicity in animals was reduced significantly by (1) gradually adapting animals to nitrate containing diets (Leng, 2008; Lee and Beauchemin, 2014), (2) adding sulphate to the nitrate containing diets (van Zijderveld et al., 2010), and (3) adding concentrate to the nitrate containing diet (Nolan et al., 2016). A number of recent *in vivo* studies using different animal species (sheep: Nolan et al., 2010; van Zijderveld et al., 2010; beef cattle: Newbold et al., 2014; Lee et al., 2017; dairy cows: Lund et al., 2014; Olijhoek et al., 2016) demonstrated effective mitigation of enteric CH_4 production with dietary nitrate addition, albeit all studies fed total mixed ration (TMR) based diets.

Limited information is available on the effect of dietary nitrate supplemented to pasture-based animals on enteric CH_4 emissions. This is feasibly due to the occurrence of nitrate in the basal diet (pasture) at fluctuating levels which can pose a risk to diet formulation. Nonetheless, the limited work undertaken in the form of an unpublished study by van Wyngaard et al. (2018) demonstrated that dietary nitrate can be fed to dairy cows grazing *Pennisetum clandestinum* dominant pasture without impinging rumen fermentation and total dry matter intake (DMI), and without reducing CH_4 emissions. However, reductions in concentrate DMI were observed feeding a high nitrate level. In the latter study, cows were supplemented with concentrate containing either 0, 11 or 23 g of nitrate/kg of dry matter (DM). Leng (2008) reported in a review that the efficacy of reducing CH_4 emissions decreases with increasing levels of nitrate fed to ruminants, which is also supported by van Zijderveld et al. (2011) who reported a linear decrease in the efficiency of reducing CH_4 production with increasing nitrate level. It could, therefore, be postulated that the lack of a treatment response in CH_4 emissions of the unpublished study of van Wyngaard et al. (2018) demonstrates that the CH_4 reducing threshold of nitrate could already have been reached by the apparent nitrate content of the pasture, or that the nitrate content margin of the diet between the control and nitrate treatments were negligible, or both. Reeves et al. (1996) reported that the NPN content of nitrogen (N)-fertilised *Pennisetum clandestinum* is higher than for temperate species such as *Lolium* spp. Given that nitrate is a NPN source, it could be hypothesised that dietary nitrate fed to dairy cows grazing predominantly *Lolium* spp. pasture could result in a reducing treatment effect on CH_4 emissions, due to the lower nitrate content of *Lolium* spp. relative to *Pennisetum clandestinum*.

Thus, the aim of the study was to determine the effect of dietary nitrate addition on CH_4 emissions, production performance and rumen fermentation of Jersey cows grazing perennial ryegrass-dominant pasture during spring. We hypothesised that CH_4 production will decrease, and that milk yield and DMI will remain unchanged with the addition of dietary nitrate.

5.3 MATERIALS AND METHODS

5.3.1 Location and Animal Ethical Clearance

This study was executed at the Outeniqua Research Farm (33°58´S, 22°25´E; Western Cape Department of Agriculture), George, South Africa, during spring of 2016 (September to November). The study area represents the temperate southern-coastal belt of South Africa, with a long-term mean annual precipitation of 732 mm, distributed throughout the year, and a mean daily maximum and minimum temperature range of 18°C to 25°C, and 7°C to 15°C, respectively. The experimental grazing area (8.55 ha) is characterised with a Podzol soil type (Swanepoel et al., 2013), with perennial ryegrass (*Lolium perenne*) sown into an existing kikuyu (*Pennisetum clandestinum*) base on an annual basis prior autumn. Before the onset of the study, ethical clearance for animal care and use was obtained from the animal ethics committee of the University of Pretoria (project number: EC078-15).

5.3.2 Animals, Experimental Design and Treatments

Thirty-two intact and eight rumen-cannulated multiparous Jersey cows were selected from the Outeniqua dairy herd, with a mean pre-experimental milk yield, days in milk (DIM), parity and body weight (BW) of 17.5 (±1.57 SD) kg/d, 104 (±48.0 SD) d, 3.9 (±1.39 SD) lactations, and 390 (±33.9 SD) kg, respectively. The experimental design was a replicated 2 × 2 Latin square design with 16 intact cow replicates and four rumen-cannulated cow replicates with treatment groups randomly allocated to one of two diets. Diets consisted of grazed pasture and supplemented concentrate, and differed by means of NPN source in the concentrate, which was either urea, or nitrate and urea, hereon referred to as the urea diet and the nitrate diet, respectively. Calcium ammonium nitrate [$5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; Yara, Oslo, Norway] was used as nitrate source. Concentrates were formulated to be isonitrogenous and isoenergetic, and was fed individually to cows at a level of 5.4 kg of DM/cow per d, in two equal portions during milking (0700 h and 1500 h). The ingredient composition of the concentrates containing

either urea, or nitrate and urea as NPN source, is shown in Table 5-1. The conservative level of the nitrate source in the nitrate concentrate was based on the pre-experimental nitrate content of the grazed pasture [3.1 (±2.65 SD) g of nitrate/kg of DM; range: 0.5 to 6.8 g of nitrate/kg of DM; n = 10]. Experimental measurement periods prolonged for 14 d. Cows were subjected to a 21-d dietary adaptation period before the onset of the measurement period. During the first 14 d of the adaptation period, cows on the nitrate diet received one of two adaptation concentrates with incremental lower levels of nitrate (33% and 66% for the first and second 7-d period, respectively) than the final nitrate concentrate treatment, followed by receiving the nitrate concentrate treatment for the last 7 d of adaptation. Cows on the urea diet only received the urea concentrate treatment for the entire 21-d adaptation period.

Table 5-1 Ingredient composition (g/kg of DM) of concentrates containing either mainly urea or nitrate as nonprotein nitrogen source.

Item[1]	Concentrate treatment	
	Urea	Nitrate
Ground maize	782	782
Soybean oilcake	40	40
Wheat bran	50	50
Molasses	50	50
Monocalcium phosphate	7	7
NaCl	5	5
$MgSO_4$	14	14.5
MgO	2	2.5
Vitamin and trace mineral premix[1]	1	1
$CaCO_3$	30	15
Nitrate source[2]	0	23.5
Urea	19	9.5

[1] Containing 4 mg of Cu/kg, 10 mg of Mn/kg, 20 mg of Zn/kg, 0.34 mg of I/kg, 0.2 mg of Co/kg, 0.06 mg of Se/kg, 6×10^6 IU of vitamin A/kg, 1×10^6 IU of vitamin D3/kg, and 8×10^3 IU of vitamin E/kg.
[2] $5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$; 750 g NO_3/kg of DM (Yara, Oslo, Norway).

5.3.3 Pasture and Grazing Management

Perennial ryegrass (73%) was the dominant pasture species of the experimental grazing area during the dietary adaptation period, followed by other grass (*Lolium multiflorum* and *Paspalum dilatatum*; 17%), white clover (*Trifolium repens*; 10%), kikuyu (4%), and broad-leaf weeds (3%). The grazing area was under permanent sprinkler irrigation and was sub-divided into 150 m x 15 m strips with electric fence. Pasture strips were N-fertilised post-grazing with limestone ammonium nitrate (containing 280 g of N/kg) at a rate of 42 kg of N/ha. Fresh pasture was allocated twice daily post-milking, with grazing areas back-fenced. Experimental cows grazed as one group in a 21-d rotational system. A target post-grazing height of 5.5 cm aboveground level was set, enforcing a strict daily herbage allowance (DHA) that was continuously adjusted throughout the study period. The degree of adjustment was determined by taking 100 pre- and post-grazing pasture height readings in a zigzag configuration for each grazing event using a rising plate meter (Jenquip folding plate pasture meter; Jenquip, Feilding, NZ). Pre- and post-grazing pasture yield (3 cm aboveground level) was estimated from pasture height readings using the following site-and-season-specific linear equation: Pasture yield (kg of DM/ha) = [103 × pasture height (rising plate meter reading)] – 261 (R^2 = 0.73).

5.3.4 Measurements

5.3.4.1 Animal performance

Experimental cows were milked twice a day at 0700 h and 1500 h during the entire experiment in a dairy parlour equipped with a 20-point swing-over Dairymaster milking machine (Dairymaster, Causeway, Co. Kerry, Ireland). Milk yield was recorded automatically with Dairymaster weigh-all electronic milk meters. Four composite morning and afternoon milk samples (24 mL) were taken during each experimental measurement period. Fat, protein, lactose and milk urea nitrogen (MUN) content were determined from each milk sample using a Milkoscan FT+ milk analyser (FOSS Analytical, DK-3400 Hillerød, Denmark), while somatic cell count (SCC) was determined using a Fossomatic FC (FOSS Analytical). Milk yield derivatives, such as energy corrected milk (ECM) and

4% fat corrected milk (FCM) were calculated using the equations of Tyrrell and Reid (1965) and Gaines (1928), respectively.

Cow BW was determined over two consecutive days at the onset and end of each experimental measurement period using a fixed weighing scale (Tru-Test EziWeigh v. 1.0 scale, 0.5 kg accuracy, Auckland, New Zealand), while cow body condition score was determined during the same time frame using the 1 to 5 scale scoring system of Wildman et al. (1982).

5.3.4.2 Dry matter intake

Individual pasture DMI from 14 intact cows per treatment group was calculated from total faecal output (FO) and indigestible neutral detergent fibre (iNDF) content of the pasture, concentrate and faeces using the equation of Cabral et al. (2014): Pasture DMI (kg/d) = [[FO (kg/d) × $iNDF_{faeces}$ (kg/kg)] – $iNDF_{concentrate}$ intake (kg/d)]/$iNDF_{pasture}$ (kg/kg). This was done for each experimental measurement period. Titanium dioxide (TiO_2; external marker) was used to determine FO (de Souza et al. (2015). Cows were dosed *per os* twice daily with gelatine capsules (size 10; Torpac Inc., Fairfield, NJ, USA) containing 3 g of TiO_2 for 10 consecutive days. Faecal samples (approximately 450 g) were collected twice daily during defecation or from the rectum on d 5 to d 10 of the TiO_2 dosing period. The remaining four intact cows of the experimental group were faecal sampled for background measures of TiO_2 that was used in the FO calculation. In succession, collected faecal samples were immediately oven dried (65°C, 72 h), pooled within-animal, milled to pass a 1 mm sieve, and analysed for TiO_2 concentration (Myers et al., 2004).

Daily pasture samples were cut (3 cm aboveground level) on the successive grazing-strips parallel to the faecal sampling period. Samples were immediately oven dried (55°C, 72 h), pooled and milled to pass a 1 mm sieve. For iNDF analysis, collected concentrate, pasture and faecal samples during the faecal sampling period were incubated *in situ* for 288 h in polyester bags (sample size to surface area ratio of 12 mg/cm^2; 07-11/5 Sefar Petex cloth, Sefar AG, Heiden, Switzerland) using three rumen-cannulated cows after the experimental study period (Krizsan et al., 2015). Following incubation, orts in the sealed polyester bags were analysed for neutral detergent fibre (NDF) according to Robertson and

van Soest (1981) by inserting the intact bags in an Ankom^{200} fibre analyser (Ankom Technology Corp., Fairport, NY) assayed with heat-stable α-amylase (protein enzyme EC 3.2.1.1; 1,4-α-D-glucan glucanohydrolase) and anhydrous sodium sulphite. The NDF values were expressed inclusive of residual ash.

5.3.4.3 Enteric methane emissions

The sulphur hexafluoride tracer gas (SF_6) technique, as described by van Wyngaard et al. (2018b) for grazing dairy cows, was used to measure CH_4 emissions from individual cows parallel to the faecal collection period (6 d) using the same 28 cows. This was done for each experimental measurement period. Permeation tubes (P&T Precision Engineering Ltd., Naas, Co. Kildare, Ireland) were loaded on-site with 3.0 (±0.14 SD) g of SF_6 gas during July 2016. Prior dosing *per os*, filled permeation tubes were calibrated over a 4-wk period in a dry incubator (Labcon, Johannesburg, South Africa) set at 39.0°C, weighed (Sartorius AG, Göttingen, Germany; 0.0001 g accuracy) every third morning to produce a 10-point regression curve (R^2 > 0.9993). Following calibration, tubes had a mean release rate of 5.1 (±0.51 SD) mg of SF_6/d (range: 4.2 to 6.1 mg of SF_6/d) one week prior dosing. Loaded and calibrated permeation tubes were dosed in gelatine capsules (Torpac Inc.).

Cow breath samples were constantly sampled, above the nostrils, into evacuated (98 kPa vacuum) polyvinyl chloride (PVC) gas-collection canisters (1700 mL) over each 24-h sampling period. An in-line flow restrictor, consisting of crimped 50 mm stainless-steel capillary tube (1/16" OD x 0.2" ID; LECO Co., Saint Joseph, MI, USA), allowed for evacuated canisters to fill to 45% capacity over the 24-h sampling period, given a fixed flow rate of approximately 0.54 mL/min. The simple back-mounted harness of van Wyngaard et al. (2018a) was used to mount the canisters. Sample canisters were replaced daily with flushed and evacuated canisters. Undiluted cow breath samples were subsampled from the sample canisters into three 12 mL glass vials (Labco Exetainer, Labco Ltd., Lampeter, Ceredigion, UK) using a piston sub-sampler (National Institute of Water and Atmosphere (NIWA) Ltd., Auckland Central, NZ). A 3-step canister flushing protocol repeated five times was implemented: (1) evacuated to 98 kPa vacuum; (2) filled with ultra-high purity N2 gas (999.99 g/kg); and (3) evacuated again to 98 kPa vacuum.

Subsampled cow breath samples were analysed (approximately 14 d after sampling) with NIWA's automated gas analyser equipped with a dual gas chromatograph (Hewlett Packard Model 6890, Palo Alto, CA, USA) containing an electron-capture detector (ECD) and a flame-ionization detector (FID) for SF_6 and CH_4 concentrations analysis, respectively. Partitioning of SF_6 and CH_4 was achieved using two parallel configured Alltech Porapak-Q 80-100 mesh columns (3.6 m × 3 mm stainless steel; Grace Davison Discovery Sciences, Deerfield, IL, USA), one for each detector. Ultra-high purity N and 10% Ar/CH_4 were used as carrier gasses at a flow rate of 30 mL/min for the FID and ECD, respectively. Operating temperatures of the FID and ECD were 250°C and 400°C, respectively. Sample loops were flushed away from the FID so the CH_4 in the ECD carrier gas was not carried through to the FID. Three standard CH_4 and SF_6 blends (NIWA) were used as calibrators prior each batch of sample vials.

The same four TiO_2 background cows were used as mobile background cows for the detection of ambient SF_6 and CH_4 concentrations. These cows did not emit SF_6 and were equipped with a similar harness to that of the SF_6 experimental cows; however the flow inlet was located on the back of the cows rather than above the nostrils'. Mobile background and SF_6 experimental cows were kept in one group at all times during grazing and milking. Collected ambient SF_6 and CH_4 concentrations of all four background cows were averaged per day to give a single estimate for all SF_6 experimental cows. This was done for each experimental measurement period. Methane production (g/d) was calculated using equation 2 from the study of Williams et al. (2011).

5.3.4.4 Rumen fermentation

A rumen fermentation study was performed with the eight rumen-cannulated cows including the following measures. Diurnal ruminal pH data were logged over a 72-h period at 10-min intervals with pre-calibrated indwelling TruTrack pH Data Loggers (Model pH-HR mark 4, Intech Instruments Ltd., Christchurch, NZ) attached to the rumen cannula. *In sacco* DM and NDF disappearance of the grazed pasture after 6, 18 and 30 h incubation were determined with the nylon bag technique of Cruywagen (2006). Subsequently, residues were analysed for NDF content (as described previously in section 5.3.4.2), and ADL content (Goering and van Soest, 1970). The NDF calculator of van Amburgh et al.

(2003) was used to calculate the rate of NDF disappearance (NDF k_d). Approximately 100 mL of ruminal fluid was collected at 8 h intervals (0600, 1400 and 2200 h) from the ventral sac using a manual vacuum pump. Subsequently, ruminal pH spot samples were immediately measured from the collected ruminal fluid with a pre-calibrated handheld pH logger (pH340i pH meter/data logger attached with a Sentix 41 pH electrode; WTW, Weilheim, Germany) before filtering through four layers of cheesecloth, and frozen in air tight containers for successive NH_3-N (Broderick and Kang, 1980) and volatile fatty acid (VFA; Filípek and Dvořák, 2009) analysis.

5.3.5 Feed Sampling and Analysis

Concentrate and pasture samples were collected weekly during each experimental measurement period, subsequently dried at 55°C for 72 h (initial DM), ground to pass through a 1 mm sieve (SMC hammer mill), and frozen pending analyses. Pasture samples were cut at 3 cm aboveground level from the successive grazing-strip. Samples were analysed for: NDF content as described previously in section 5.3.4.2; ether extract, DM, ash and crude protein (CP) content (N content determined using a LECO Trumac™ N Determinator, LECO Corporation, Saint Joseph, MI, USA) according to procedures of AOAC (2000; methods 920.39, 934.01, 942.05, and 968.06, respectively); acid detergent fibre according to Goering and van Soest (1970) using the Ankom^{200} fibre analyser; gross energy content (MC–1000 modular calorimeter, operator's manual); mineral composition and nitrate content according to the procedures of AgriLASA (1998, method 6.1.1; and 2004, respectively); and *in vitro* organic matter digestibility (Tilley and Terry, 1963) using rumen fluid from a rumen-cannulated SA Mutton Merino ram fed good-quality lucern hay. Metabolisable energy (ME) was calculated (MAFF, 1984).

5.3.6 Statistical Analysis

Individual production variables measured daily (milk yield, DMI, and CH_4 parameters) and weekly (milk composition parameters), and rumen fermentation variables measured per time interval were averaged within-cow per experimental period. The average successful collection rate of cow breath samples from 384 canisters across the two experimental periods were 82%. Lost samples were mainly due to flow restrictor blockages and broken sampling lines during the 24-h collection periods. Methane data were subjected to outlier analysis by means of the modified Z-score as described by Berndt et al. (2014), where data associated with 'modified Z-scores' of >3.5 (absolute value) were labelled as outliers.

Milk parameters and body condition parameters of cows measured during the overall experimental measurement period were analysed with analysis of variance to test for differences between treatment effects as a replicated 2 x 2 Latin square design, repeated 16 times. Individual DMI and CH_4 emissions, and rumen fermentation measures were statistically analysed in a similar fashion, but repeated 14 times and four times, respectively. Normality tests were performed on all datasets and residuals were acceptably normal with homogeneous treatment variances, except for SCC which were log (base 10) transformed, and time spent below ruminal pH that was Poisson distributed and thus analysed with generalised linear model analysis to test for differences between treatment effects. Treatment means were compared using Tukey's least significant difference test at the 5% level of significance (Snedecor and Cochran, 1980). Data were analysed using the statistical program GenStat (Payne et al., 2014).

5.4 RESULTS

5.4.1 Feed Composition and Pasture Management

Nitrate was present in the urea concentrate at a minimal level of 0.3 g of nitrate/kg of DM compared with 15.2 g of nitrate/kg of DM in the nitrate concentrate (Table 5-2). Chemical composition, and pre- and post-grazing measurements of the pasture were fairly

similar between the two measurement periods (Table 5-2 and Table 5-3). Average pasture intake as measured with a rising plate meter above 3 cm of ground level was 9.6 kg of DM/cow per d, given an average daily herbage allowance (DHA) of 14.2 kg of DM/cow per d and pasture DM yield of 2.3 t/ha (Table 5-3). Cows consumed on average 68% of the offered DHA leaving behind an average post-grazing height of 5.5 cm.

Table 5-2 Chemical composition (mean ± SD) of concentrates containing either mainly urea or nitrate, and of the pasture offered (per measurement period).

Item[1]	Concentrate treatment (n = 5)		Pasture[2] (n = 5)	
	Urea	Nitrate	Period 1	Period 2
Initial DM (%)	89.4 ± 0.42	89.3 ± 0.17	13.9 ± 0.10	14.3 ± 0.67
DM composition (g/kg of DM)				
Ash	74.5 ± 3.40	83.6 ± 7.30	118 ± 3.3	116 ± 3.7
CP	131 ± 2.0	148 ± 7.0	218 ± 6.0	220 ± 10.0
Nitrate	0.31 ± 0.112	15.2 ± 0.98	7.27 ± 0.494	7.24 ± 1.044
NDF	99.7 ± 6.54	89.1 ± 1.50	475 ± 25.7	510 ± 9.8
ADF	28.4 ± 7.91	22.0 ± 2.02	278 ± 11.8	281 ± 5.7
EE	13.7 ± 0.79	12.1 ± 1.10	35.2 ± 3.76	38.4 ± 1.00
*IV*OMD	953 ± 12.6	969 ± 4.8	895 ± 17.6	875 ± 30.1
GE(MJ/kg of DM)	17.1 ± 0.05	16.7 ± 0.20	17.7 ± 0.11	17.9 ± 0.19
ME (MJ/kg of DM)	13.7 ± 0.19	13.6 ± 0.16	12.9 ± 0.22	12.7 ± 0.50
Mineral composition				
Ca (g/kg of DM)	15.4 ± 0.65	19.0 ± 2.22	4.70 ± 0.430	4.42 ± 0.296
P (g/kg of DM)	5.72 ± 0.119	5.16 ± 0.074	5.03 ± 0.257	4.68 ± 0.313
Mg (g/kg of DM)	4.80 ± 0.281	6.88 ± 0.681	3.49 ± 0.142	3.56 ± 0.193
K (g/kg of DM)	8.22 ± 0.147	8.79 ± 0.292	31.3 ± 2.06	30.3 ± 2.43
Na (g/kg of DM)	2.64 ± 0.252	5.63 ± 1.641	21.9 ± 2.70	22.0 ± 4.8
Mn (mg/kg of DM)	93.2 ± 3.76	182 ± 38.2	36.0 ± 5.28	34.4 ± 5.18
Cu (mg/kg of DM)	29.8 ± 1.76	53.0 ± 8.81	7.55 ± 0.792	8.29 ± 0.738
Fe (mg/kg of DM)	192 ± 12.4	172 ± 6.3	226 ± 66.4	286 ± 22.4
Zn (mg/kg of DM)	141 ± 3.7	191 ± 32.7	44.9 ± 5.10	52.9 ± 4.00

[1] DM–dry matter; CP–crude protein; NDF–neutral detergent fibre; ADF–acid detergent fibre; EE–ether extract; *IV*OMD–*in vitro* organic matter digestibility; GE–gross energy; ME–metabolisable energy.
[2] Pasture – perennial ryegrass (*Lolium perenne*) dominant.

Table 5-3 Pre- and post-grazing measurements of the experimental perennial ryegrass dominant pasture (mean ± SD of each measurement period).

Item[1]	Period 1	Period 2
Pasture height (cm)		
Pre-grazing	12.3 ± 3.08	12.2 ± 1.10
Post-grazing	5.47 ± 0.851	5.53 ± 0.748
Pasture yield (kg of DM/ha)[2]		
Pre-grazing	2280 ± 634.5	2256 ± 225.7
Post-grazing	865 ± 175.2	879 ± 154.1
DHA (kg of DM/d)	14.1 ± 2.76	14.3 ± 3.54
Daily grazed area (m^2/cow)	64.7 ± 20.61	63.0 ± 14.23
Pasture removed (kg of DM/d)	9.78 ± 3.163	9.33 ± 2.888

[1] DM–dry matter; DHA–daily herbage allowance.
[2] Pasture yield (kg of DM/ha) = (103 × rising plate meter reading) – 261 (R^2 = 0.73); estimated 3 cm aboveground level using a rising plate meter.

5.4.2 Milk yield, Milk Composition and Cow Condition

Milk yield and derivatives (FCM and ECM) were unaffected (P>0.05) by treatment (Table 5-4). However, a number of treatment changes were observed in milk composition. Milk fat content tended (P=0.067) to be higher for the nitrate diet compared with the urea diet, but milk fat yield remained the same (P>0.05). The milk fat content results led to a tendency for the milk protein to fat ratio to be lower (P=0.059) for the nitrate diet compared with the urea diet, because milk protein content remained unchanged between treatments (P>0.05). The higher (+0.6 g/kg; P=0.014) milk lactose content observed for cows on the nitrate diet resulted in higher (+2 g/kg; P=0.027) total milk solid content for the nitrate diet compared with the urea diet. Milk urea nitrogen was higher (+1 mg/dL; P<0.001) for cows on the nitrate diet than for cows on the urea diet.

Table 5-4 Milk production (mean of study period) and cow condition of early lactation Jersey cows grazing perennial ryegrass dominant pasture during spring fed concentrate containing either mainly urea or nitrate (mean of the two measurement periods).

Number of cows	32	32		
Item[1]	Concentrate treatment[2]		SEM[3]	P-value
	Urea	Nitrate		
Milk yield (kg/d)	19.0	18.9	0.12	0.39
FCM yield (kg/d)	21.6	21.8	0.22	0.46
ECM yield (kg/d)	21.6	21.8	0.19	0.48
Milk fat (g/kg)	49.0	50.7	0.59	0.067
Milk protein (g/kg)	36.9	36.8	0.13	0.39
Milk protein to milk fat ratio	0.76	0.73	0.010	0.059
Milk lactose (g/kg)	46.7[b]	47.3[a]	0.14	0.014
Total milk solids (g/kg)	133[b]	135[a]	0.59	0.027
MUN (mg/dL)	11.6[b]	12.6[a]	0.10	<0.001
Log_{10} SCC	2.09	2.06	0.048	0.68
Milk fat yield (kg/d)	0.93	0.95	0.012	0.25
Milk protein yield (kg/d)	0.70	0.69	0.005	0.19
Milk lactose yield (kg/d)	0.89	0.89	0.007	0.83
BW change (kg)	+10.5	+8.2	2.84	0.58
BCS change (scale 1 to 5)	0.09	0.13	0.025	0.21

[a,b,c] Row means with different superscripts differ significantly at P<0.05.
[1] FCM–4% fat corrected milk; ECM–energy corrected milk; Total milk solids = milk fat + milk protein + milk lactose; MUN–milk urea nitrogen; SCC–somatic cell count; BW–body weight; BCS–body condition score.
[2] Concentrate fed at 5.4 kg (dry matter basis)/cow per day split in two equal portions during milking, containing either 0.3 or 15.2 g of nitrate/kg of dry matter.
[3] SEM–standard error of mean.

5.4.3 Dry Matter Intake and Enteric Methane Emissions

Pasture and total DMI were unaffected (P>0.05) by treatment (Table 5-5). Cows had an average pasture and total DMI of 13 and 18 kg/cow per d, respectively. Methane emissions were also unaffected (P>0.05) by treatment. Cows had an average CH_4 production (g/d), CH_4 yield (g/kg of DMI) and CH_4 intensity (g/kg of ECM) of 403, 24 and 19. Milk parameters measured during the CH_4 measurement period reflected that of the milk parameters averaged across the whole study period. However, milk fat content was at this point statistically higher (+2.3 g/kg; P=0.005) for cows on the nitrate diet compared with cows on the urea diet.

Table 5-5 Body weight, faecal output, dry matter intake, methane emissions and milk production (mean of methane measurement period) of early lactation Jersey cows grazing perennial ryegrass dominant pasture during spring fed concentrate containing either mainly urea or nitrate (mean of the two measurement periods).

Number of cows	28	28		
Item[1]	Concentrate treatment[2]		SEM[3]	P-value
	Urea	Nitrate		
BW (kg)	390	389	0.9	0.42
FO (kg of DM/d)	3.66	3.49	0.174	0.51
Intake				
Pasture DMI (kg/d)	12.7	12.4	0.62	0.77
Total DMI (kg/d)	18.1	17.8	0.62	0.76
NDF intake as % of BW	1.74	1.72	0.077	0.80
DMI as % of BW	4.67	4.61	0.16	0.82
GEI (MJ/d)	318	311	11.1	0.67
MEI (MJ/d)	236	232	7.89	0.73
CH_4 emissions				
CH_4 production (g/d)	400	405	14.5	0.81
CH_4/DMI (g/kg)	23.2	24.3	0.98	0.47
CH_4/milk yield (g/kg)	21.4	21.9	0.73	0.61
CH_4/ECM (g/kg)	18.9	18.8	0.64	0.93
CH_4 energy (MJ/d)	22.1	22.4	0.802	0.80
Y*m* (%)	7.29	7.67	0.312	0.41
Milk yield (kg/d)	19.0	18.7	0.17	0.24
ECM (kg/d)	21.5	21.6	0.24	0.64
Milk fat (g/kg)	48.5[b]	50.8[a]	0.47	0.005
Milk protein (g/kg)	36.9	36.5	0.14	0.11
Milk lactose (g/kg)	47.1	47.4	0.19	0.30

[a,b,c] Row means with different superscripts differ significantly at P<0.05.
[1] BW–body weight; FO–faecal output; DM–dry matter; DMI–dry matter intake; NDF–neutral detergent fibre; GEI–gross energy intake; MEI–metabolisable energy intake; CH_4–methane; ECM–energy-corrected milk; FCM–fat-corrected milk; CH_4 energy = (55.22 MJ*CH_4 g/d)/1000; Y*m*–methane energy per gross energy intake.
[2] Concentrate fed at 5.4 kg (dry matter basis)/cow per day split in two equal portions during milking, containing either 0.3 or 15.2 g of nitrate/kg of dry matter.
[3] SEM–standard error of mean.

5.4.4 Rumen Fermentation

Diurnal ruminal pH showed a cyclic drop in pH after feeding of concentrate in the morning and afternoon for both treatments (Figure 5-1). Cows on the nitrate diet showed

intermittent higher (P<0.05) ruminal pH values, compared with cows on the urea diet, throughout the 24-h period, which seems to be more prevalent post-afternoon feeding of concentrate.

However, mean diurnal pH was unaffected (P>0.05) by treatment (Table 5-6). Conversely, spot samples of ruminal pH from extracted ruminal fluid tended (P=0.091) to be higher for cows on the nitrate diet compared with cows on the urea diet. Ruminal NH_3-N, VFA concentrations, and *in sacco* DM and NDF disappearance of the grazed pasture were unaffected (P>0.05) by treatment.

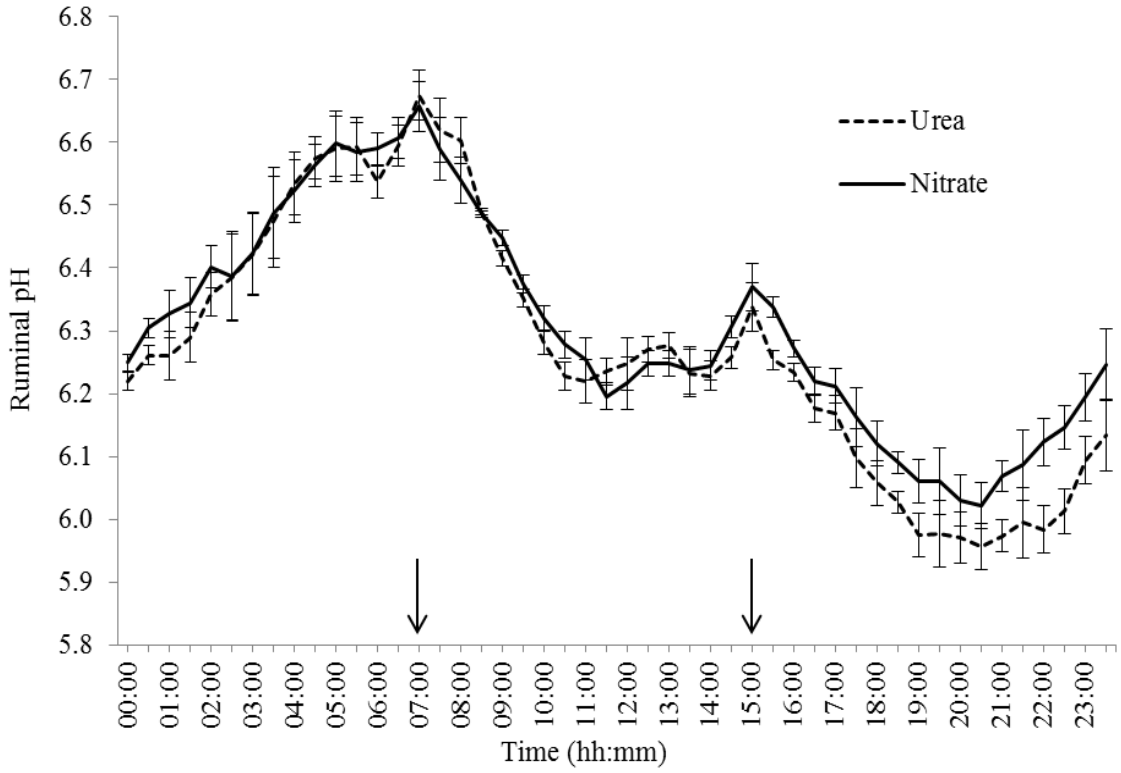

Figure 5-1 Diurnal ruminal pH pattern of early lactation Jersey cows grazing perennial ryegrass dominant pasture during spring fed concentrate containing either urea or nitrate (mean of the rumen measurement periods; n = 8). Concentrate fed at 5.4 kg (dry matter basis)/cow per day split in two equal portions during milking, containing either 0.3 or 15.2 g of nitrate/kg of dry matter. Error bars indicate standard error of mean and arrows indicate when concentrate was fed.

Table 5-6 Ruminal pH, volatile fatty acid profile, NH_3-N concentration, and *in sacco* disappearance of the grazed pasture of early lactation Jersey cows grazing perennial ryegrass dominant pasture during spring fed concentrate containing either mainly urea or nitrate (mean of the rumen measurement periods).

Number of cows	8	8		
Item[1]	Concentrate treatment[2]		SEM[3]	P-value
	Urea	Nitrate		
Diurnal pH (over 72 h)	6.28	6.31	0.025	0.41
Spot sample pH	5.97	6.05	0.021	0.091
Time below (h)				
pH 5.8	2.06	0.69	0.753	0.24
pH 6.0	3.75	3.13	1.81	0.81
pH 6.2	7.50	7.38	1.91	0.96
pH 6.4	15.9	14.6	1.48	0.53
pH 6.6	21.1	21.3	1.20	0.94
NH_3-N (mg/dL)	17.9	17.6	0.50	0.72
Total VFA (m*M*/L)	129	122	4.5	0.38
Acetic (m*M* %)	82.2	82.6	0.41	0.48
Propionic (m*M* %)	10.9	10.6	0.21	0.39
Butyric (m*M* %)	5.92	5.88	0.152	0.87
Isobutyric (m*M* %)	0.27	0.24	0.017	0.33
Valeric (m*M* %)	0.30	0.27	0.025	0.38
Isovaleric (m*M* %)	0.33	0.27	0.027	0.24
Caproic (m*M* %)	0.095	0.089	0.0042	0.37
DM disappearance (coeff.)				
6 h	0.46	0.45	0.009	0.73
18 h	0.71	0.72	0.009	0.59
30 h	0.85	0.84	0.006	0.48
NDF disappearance (coeff.)				
6 h	0.23	0.22	0.015	0.59
18 h	0.56	0.57	0.013	0.80
30 h	0.76	0.75	0.008	0.39
NDF k_d (per hour)				
6 h	0.051	0.048	0.0038	0.62
18 h	0.061	0.065	0.0028	0.44
30 h	0.077	0.072	0.0030	0.34
Mean	0.063	0.061	0.0023	0.67

[a,b,c] Row means with different superscripts differ significantly at P<0.05.
[1] NH_3-N–ammonia nitrogen; VFA–volatile fatty acid; DM–dry matter; NDF–neutral detergent fibre; NDF k_d–rate of neutral detergent fibre disappearance.
[2] Concentrate fed at 5.4 kg (dry matter basis)/cow per day split in two equal portions during milking, containing either 0.3 or 15.2 g of nitrate/kg of dry matter.
[3] SEM–standard error of mean.

5.5 DISCUSSION

This study aimed to evaluate the effect of supplementing NPN, using urea or, urea and nitrate as NPN source, to early lactation dairy cows grazing predominantly perennial ryegrass pasture during spring on CH_4 emissions, DMI, milk production, and rumen fermentation. The major findings of this study were that CH_4 emissions, DMI, milk yield and rumen fermentation were unchanged, while total milk solids were the highest for the nitrate treatment.

Probably the main reason for a lack of treatment response in CH_4 emissions is that this study failed to exert a significant margin in total nitrate intake between the urea and nitrate treatment diets being 5.2 and 9.7 g of nitrate/kg of DM, respectively, or 0.24 and 0.44 g of nitrate/kg of BW. This was imposed by the unexpected high nitrate content (7.3 g/kg of DM) of the grazed pasture during the experimental measurement periods, especially when the pre-experimental nitrate content of the grazed pasture was at a mere 3.1 g/kg of DM. This phenomenal increase is unclear, particularly since the N-fertiliser source and application rate remained unchanged from the pre-experimental measurements through to the experimental measurements. Additionally, pasture quality (CP, ME and NDF content) and botanical composition of the experimental pasture was similar to that of perennial ryegrass pasture described by van der Colf et al. (2015) from the same research farm in South Africa. This apparent nitrate accumulation in the pasture could have resulted from moisture stress. A dry spell was experienced during the dietary adaptation period followed by cool and overcast conditions during the experimental measurement periods. In agreement with this, Goh and Haynes (1986) reported that New Zealand pastures accumulated nitrate under cloudy and cool conditions succeeding dry spells. This demonstrates the sensitivity of nitrate content in pasture towards plant stress factors and, therefore, underlines the risk when supplementing nitrate to pasture-based animals even when pasture is under permanent irrigation and N-fertiliser is applied at moderate levels.

The average enteric CH_4 production obtained in the current study (403 g/d) were generally higher than in previous studies that evaluated enteric CH_4 emissions from grazing dairy cows. Jiao et al. (2014) and Muñoz et al. (2015) reported average enteric CH_4 production values of 277 and 355 g/d, respectively, from Holstein-Friesian dairy cows

grazing *Lolium perenne* pasture with average individual DMI of 15 and 19 kg/cow per d, respectively. In comparison to Jiao et al. (2014), the higher enteric CH_4 production in the current study may be rationalised by the higher DMI (18 kg/cow per d) attained, as DMI is the most important predicting factor for enteric CH_4 emissions (Niu et al., 2018). Whereas, when compared with Muñoz et al. (2015) who reported a similar DMI, the higher enteric CH_4 production in the current study may be explained by the higher NDF content in the pasture (49% *vs.* 45%), as more CH_4 emissions is emitted when diet NDF:starch ratio increases (Moe and Tyrrell, 1979). Additionally, Bell et al. (2014) reported that CH_4 emissions between cows can vary substantially even when fed the same diet at the same intake. Average CH_4 energy per GE intake (Y*m*) in the current study (7.5%) is slightly higher than the typically Ym range of 6 to 7% for forage-based diets (Johnson and Johnson, 1995), but lower than the average Y*m* (9.2%) reported by Dall-Orsoletta et al. (2016). The latter study fed F1 Holstein × Jersey cows partial TMR with restricted access to *Lolium multiflorum* pasture.

Hypothetically, a dietary nitrate content difference of 4.5 g/kg of DM could result in a 12% reduction in CH_4 yield using the prediction equation of Lee and Beauchemin, (2014): CH_4 yield (g/kg of DMI = –8.3 × nitrate (g/kg of BW) + 15.2; R^2 = 0.80. However, this was not found in our study indicating that the prediction equation could not be extrapolated to pasture-based systems. Although blood methaemoglobin was not measured during this study, no sub-clinical signs of nitrate poisoning (losses of weight and milk production, or non-infectious abortions in dairy cattle; Bolan and Kemp, 2003) were observed during the experimental periods. This was merely because total nitrate intake (0.24 and 0.44 g of nitrate/kg of BW for the urea and nitrate treatment, respectively) was far below the levels that were proposed to cause sub-clinical nitrate toxicity in both unadapted (0.69 to 0.94 g of nitrate/kg of BW) and adapted animals (7 to 9 g of nitrate/kg of BW) using the regression equation of Lee and Beauchemin, (2014): Y = 4.2 × nitrate (g/kg of BW per day) + 0.4 (R^2 = 0.76), where Y is blood methaemoglobin %. The threshold for subclinical nitrate toxicity was proposed to be at blood methaemoglobin levels of 30% to 40% (Bruning-Fann and Kaneene, 1993).

Milk lactose content is positively related to milk yield (Linzell and Peaker, 1971). This was not observed in the current study as milk yield was unchanged despite the

increase in milk lactose content (+0.6 g/kg) for the nitrate treatment. The occurrence of this increase is unclear as the nitrate source in this study did not alter VFA proportions which are precursors for milk composition components, therefore the small extent thereof makes it negligible. Previous studies supplementing nitrate to dairy cows (van Zijderveld et al., 2011; Klop et al., 2016) did not report an increase in MUN concentration which contradicts the findings of the current study. Urinary N excretion can be estimated using MUN concentration (Kohn et al., 2002). Therefore the increase in MUN suggests that cows supplemented with nitrate and urea excreted more urinary N than cows supplemented with urea only as NPN source. Although ruminal NH_3-N concentration was unaffected by treatment, it could be postulated that excess NH_3, derived from reduced nitrate, or unreduced nitrate was absorbed through the rumen wall into the bloodstream and forming MUN or excreted in urine or both. Pasture-based animals are accustomed to NPN sources acquired from grazed pasture, and therefore surplus NPN is cycled into MUN or excreted in the urine. This suggests that nitrate would unlikely have an effect on enteric CH_4 emissions from cows grazing pasture already containing high levels of NPN sources such as nitrate.

Milk fat content is positively related to acetic acid concentration in the rumen (Seymour et al., 2005). Unaffected rumen fermentation results (VFA profile and ruminal DM and NDF disappearance) of the current study failed to support the observed increase in milk fat content (+2.3 g/kg) for the nitrate treatment. Although Klop et al. (2016) did not report a dietary nitrate treatment effect on milk fat content of dairy cows, the authors did report increased levels of C14:0 iso and C15:0 iso in milk fat, which they suggested is indicative of an increased abundance of fibrolytic bacteria. Conversely, the authors also found that nitrate reduced total-tract apparent fibre digestion. This indicates that the effect of nitrate on milk fat content needs further investigation for clarification. Furthermore, irregular increases in ruminal pH throughout the diurnal pattern for the nitrate treatment may by indicative of increases in hydrogen. Heightened hydrogen levels in the rumen with nitrate supplementation were previously reported by van Zijderveld et al. (2011), Lund et al. (2014) and Olijhoek et al. (2016) with the reason thereof not yet established. The lack of treatment response on rumen fermentation of dairy cows supplemented with nitrate has been previously reported on (Lund et al., 2014; Olijhoek et al., 2016).

This study demonstrated that nitrate is not an effective enteric CH_4 mitigation strategy for dairy cows grazing perennial ryegrass. The increase in MUN observed in this study is indicative that N was fed in excess. This may contribute to other N losses that end up in manure and emitted as N_2O, which has more than 9-times the global warming potential than that of CH_4 over a 100 y period (Myhre et al., 2013).

5.6 CONCLUSIONS

Nitrate as NPN source supplemented to grazing dairy cows did not reduce CH_4 emissions nor did it affect DMI or milk yield. Milk fat content increased when nitrate was supplemented. Rumen fermentation was not adversely affected. Future research is encouraged to evaluate the effect of nitrate supplementation on milk fat content.

5.7 ACKNOWLEDGEMENTS

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CHAPTER 6

Technical note: A simple back-mounted harness for grazing dairy cows to facilitate the sulphur hexafluoride tracer gas technique

6.1 ABSTRACT

We describe here a cattle harness to attach a gas collection vessel to facilitate the sulphur hexafluoride (SF_6) tracer gas technique. The harness consists of two major components: (1) a lightweight, robust body fabricated from an equine surcingle or lunge roller with padded thoracic trapezius pressure points, a bespoke shaping shaft for spine support, and adjustable buckles on both sides; and (2) an elastic flank-strap to prevent the harness from dislodging. The spine support consists of stainless steel laminated with carbon fibre. This support minimises the contact area with the animal's skin, relieves the spine area of pressure, and creates free flow of ambient air below the platform, reducing sweat accumulation and hence preventing skin lesions. The harness weighs approximately 1.2 kg, allows for attachment of two gas collection vessels (animal and background sample), and is cost effective.

Key words: sulphur hexafluoride (SF_6); methane measurement; enteric CH_4; equipment; harness

6.2 TECHNICAL NOTE

Enteric methane emissions from individual grazing ruminants can be measured using the sulphur hexafluoride (SF_6) tracer gas technique developed by Zimmerman (1993) and first adopted by Johnson et al. (1994). Since 1994, various implementations of the original technique have been published in more than 120 peer-reviewed papers. In an attempt to standardise the SF_6 technique, a few guidelines on the use thereof have been made available over time (Johnson et al., 2007; Berndt et al., 2014; Williams et al., 2016), with the latest modification for dairy cattle described in detail by Deighton et al. (2014). These

guidelines concentrated profoundly on the fundamental elements of the SF_6 technique, such as the slow-release device (permeation tube) and sampling line with flow restrictor and gas collection vessel (sample and background). The gas collection vessel has changed from a stainless steel sphere suspended by a neck strap attached to the halter apparatus (Johnson et al., 1994) to a V- or U-shaped neck yoke moulded from polyvinyl chloride (PVC) pipe (Johnson et al., 2007) and, most recently, to a stainless steel or PVC cylinder fitted to the animal's back (O'Neill et al., 2011; Deighton et al., 2013, 2014). The mounting position of the gas collection vessel depends mainly on the species and breed (size and temperament) as well as the operating environment (outdoor or indoor) and the available resources to manufacture the vessel. For example, the neck position for the gas collection vessel will function for most animals when operating outdoors, whereas it will not be functional in a milking parlour or feed stall equipped with a baling system. The back-mounting options for the gas collection vessel are, however, usually described superficially, often not cost effective, and not standardised. In our opinion, the position and quality—in terms of support, minimal skin contact area, and pressure points—of the mount on the animal are critical as these factors will affect animal welfare and the number of representative gas samples lost.

This note presents a cost-effective, robust, back-mounted harness with minimum skin contact area for grazing dairy cows that facilitates the SF_6 technique for measurement of enteric methane emissions. We hypothesise that grazing dairy cows equipped with this novel harness will not show signs of skin lesions on the spine area or behind the thoracic limb. Although the harness described in this note applies to dairy cows, the apparatus could be adapted for use in other ruminants as well. Institutional animal care and use was obtained from Western Cape Department of Agriculture (Elsenburg, South Africa) before commencement of the study, and unnecessary discomfort to the animals was avoided at all times.

The harness consists of two major components: (1) a lightweight, robust body fabricated from an equine surcingle or lunge roller with padded thoracic trapezius pressure points, a bespoke shaping shaft for spine support, and adjustable buckles on both sides, which act as a platform for gas collection vessel attachment; and (2) an elastic flank-strap to prevent the harness from sliding over the neck of the animal. The padded surcingle used

is commercially available and is specifically designed to relieve pressure on the spine and to avoid sideways movement of the harness. The surcingle is equipped with attachment rings running from the ribcage up to the spine area and usually has a girth range of 160 to 220 cm. Nylon is recommended over leather for the surcingle because of the lighter weight and enhanced breathability, keeping sweat accumulation to a minimum. The trapezius padding is covered by perforated neoprene material to ensure breathability and comfort (Figure 6-1). We found that the standard padded surcingle does not relieve sufficient pressure on the spine due to the Jersey cow's pointed thoracic spinous process, which is more profound in a grazing system compared with a total mixed ration system where energy supply is not limiting and body condition is improved.

Figure 6-1 Harness body showing the perforated neoprene padding with built-in support shaft to ensure breathability and comfort to the trapezius area of the cow while acting as a platform for attachment of the gas collection vessel.

As observed in our previous SF_6 trials, more than 40% and 20% (n = 72) of pasture-based Jersey cows equipped with the standard padded surcingle without protective felt wrapping, covering an average distance of 800 m twice daily around milking for six consecutive days, showed signs of skin lesions on the spine area and behind the thoracic limb, respectively, ranging from slight to severe cases. Unfortunately, exact values of skin lesion incidences from other research establishments for comparison purposes are difficult to obtain due to the sensitive nature thereof.

To alleviate the problem, a U-shaped trapezoidal support shaft was crafted from stainless steel rod (6 mm diameter) laminated with one layer of stringed carbon fibre per side to create a flat area with rounded edges. The lengths of the sides are 170 mm, the top base 70 mm, and the width of the laminated shaft 40 mm (Figure 6-2). The support shaft weighed approximately 177 g. The inner base angle of the support shaft can range from 120° for cows with body condition score (BCS) <2.5 to 150° for cows with BCS >3.0. The BCS system used was the 5-point scale developed by Wildman et al. (1982).

Figure 6-2 The U-shaped trapezoidal support shaft crafted from stainless steel rod laminated with stringed carbon fibre.

The shape and size of the support shaft was based on a gypsum mould of the thoracic vertebrae area of a Jersey cow with a BCS of 2.0. The support shaft is inserted within the surcingle between the two nylon layers, above the trapezius padding, and stitched secure. This support relieves the spine area of any possible base pressure imposed by the weight of the gas collection vessel, minimises the skin contact area (alleviating skin lesions), creates free flow of ambient air below the base (reducing sweat accumulation that attract flies), and creates a platform for attachment of the gas collection vessel.

To prevent the harness body from dislodging and sliding over the neck of the animal, a single elastic band (dimensions: 25 to 40 mm wide, 1 to 2 mm thick, and approximately 2 m long unstretched, depending on the girth and length of the cow) is connected caudally via attachment rings to the mid-rib area on both sides of the harness body. The elastic band is connected at 30% stretch and should run over the flank area of the animal under the tail (Figure 6-3).

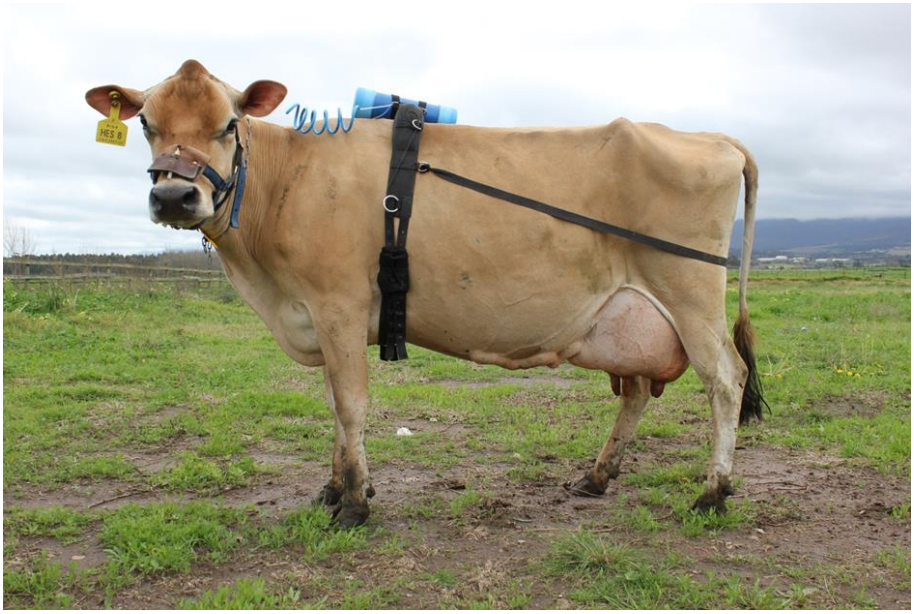

Figure 6-3 A Jersey cow equipped with a simple back-mounted harness for gas collection vessel attachment to facilitate the sulfur hexafluoride tracer gas technique. Adjustable buckles and elastic band over the flank avoid dislodgment of the harness.

The harness body is fitted so that the adjustable buckles are at equal heights, >70 mm above the olecranon tuber area of the thoracic limb, minimising skin lesions (Figure 6-4a). The harness body should be tightened to prevent excessive sideways movement but allowing restricted hand movement under the body of the harness body in the mid-rib area. To further preclude the possibility of skin lesions, we wrapped the adjustable buckles with a double layer of felt material fixed with elastic bands (Figure 6-4b).

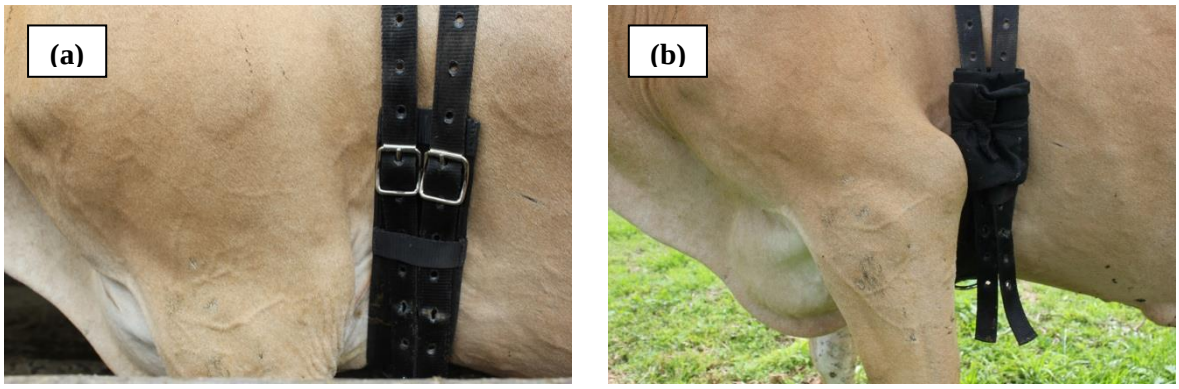

Figure 6-4 (a) Placement of the adjustable buckles >70 mm above the olecranon tuber area of the thoracic limb (b) wrapped with double layer of felt material to minimise skin lesions.

The gas collection vessel is positioned recumbent, parallel to the animal's spine on the platform created by the harness body. The vessel is fixed with two double-sided Velcro (London, UK) strips, cranially and caudally, via a cable tie looped through one of the attachment rings available on the harness body (Figure 6-5). The double-sided Velcro strips allow quick replacement of gas collection vessels, while providing a robust, cost-effective attachment.

This novel harness has recently been implemented in two SF_6 trials that, combined, consisted of 68 pasture-based, lactating Jersey cows covering the same distance for the same duration under similar conditions as the previously mentioned SF_6 trials. During the trial, none of the harnesses dislodged and no signs of skin lesions on both the spine and thoracic limb area were detected after completion of the trial. Hence, we accept our hypothesis that this novel harness will not cause skin lesions in grazing dairy cows.

Figure 6-5 The gas collection vessel is attached with two double sided Velcro (London, UK) strips via a cable-tie looped through one of the attachment rings available on the harness body, facilitating quick replacement of the vessel.

The complete harness, excluding the gas collection vessel, weighs approximately 1.2 kg and could cost less than US$70. The harness allows for attachment of two gas collection vessels, thereby allowing for individual on-cow background sampling. We recommend that the animal be equipped with the harness without the collection vessel at least 2 d before the sampling period to allow the animal to adapt to the harness. This harness has been developed over a series of SF_6 trials to the point where it functions successfully while not causing any skin lesions, and therefore satisfactory from an animal welfare point of view.

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CHAPTER 7

Short Communication: Comparison of the laser methane detector and SF_6 technique to measure enteric methane emissions from grazing dairy cows

7.1 ABSTRACT

There is a need for an inexpensive high-throughput technique to measure methane (CH_4) emissions from individual animals to identify high milk producing, low CH_4 emitting animals. The aim of this paper was to compare methane (CH_4) emission rates as measured by the LMD to the sulphur hexafluoride tracer gas (SF_6) technique from lactating dairy cows grazing pasture and to evaluate the practicality of the LMD operating protocol under grazing conditions in the temperate coastal area of South Africa. Methane production was determined from six lactating Jersey cows on pasture using both techniques. Methane output data from the LMD had a higher (60 *vs*. 40%) between-cow coefficient of variation (CV) compared with data obtained from the SF_6 technique. This was ascribed to the sensitivity of the LMD to ambient conditions, animal movement while grazing and time of measurement. Methane production as measured by the SF_6 technique (348 g/d) was higher compared with the LMD technique (82.6 g/d). Results from this study indicated that the LMD underestimated CH_4 production by 76%. Findings of this study indicate that there is a need to improve the LMD operating protocol and scale-up factors to accurately convert CH_4 concentration (ppmv.m) to CH_4 production (g/d).

Key words: *in vivo*; Jersey cow; LMD; pasture; sulphur hexafluoride tracer gas; ERUCT technique

7.2 INTRODUCTION

Recently, increasing evidence for climate change has amplified the need to verify national greenhouse gas (GHG) inventories and to validate on-farm GHG mitigation strategies. Globally, the livestock sector emits approximately 7.1 Gt of carbon-equivalent

per annum, with enteric methane (CH_4) from ruminants constituting approximately 39% of the sectors emissions (Gerber et al., 2013). Traditionally, the majority of studies concerned with animal energetics and daily CH_4 production have utilised respiration chambers and enclosures as CH_4 measurement tools. However, respiration chambers restrict normal animal behaviour, such as animal movement, diet selection, and animal interaction with the natural environment and other animal peers (Pinares-Patiño et al., 2011). The use of chambers also limits the number of experimental animals per trial and can be particularly costly and labour intensive (Hammond et al., 2016). Consequently, the demand to measure enteric CH_4 from individual animals within their natural production environment arose in response and the sulphur hexafluoride tracer gas (SF_6) technique was developed by Zimmerman (1993) and later modified by Deighton et al. (2014). The concept of the SF_6 technique is that an inert tracer gas (SF_6) is released in the rumen at a known rate and sampled alongside the gas of interest (CH_4) from the nasal cavity area over a 24 h period, and this is repeated over a minimum of five consecutive days. The SF_6 technique has extensively been evaluated by several comparison studies with respiration chambers (Boadi et al., 2002; Grainger et al., 2007; Pinares-Patiño et al., 2008, 2011; Muñoz et al., 2012; Deighton et al., 2014). Hammond et al. (2016) summarised in a review that mean CH_4 emissions measured by the SF_6 technique can differ by 5-10% (lower or higher) for the same animals measured by respiration chambers and may present larger within- and between-animal variation relative to respiration chambers (Grainger et al., 2007; Pinares-Patiño et al., 2008). In an attempt to identify and address factors affecting the accuracy and precision of the SF_6 technique, a few guidelines on improvements and modifications have consequently been made (Johnson et al., 2007; Berndt et al., 2014; Williams et al., 2016). These SF_6 technique improvements have resulted in a between-animal CV for CH_4 emissions of 6.5%, similar to that determined using respiration chambers (7.5%; Deighton et al., 2014). For that reason, the recent improved SF_6 technique is one of the most reliable methods to determine CH_4 emissions over a 24 h period from individual grazing animals.

In an attempt to meet the demand of a higher throughput in terms of number of individual animal measurements to verify GHG inventories, a variety of short-term measurement techniques based on spot measurement of exhaled CH_4 at specified time points, were developed (Hammond et al., 2016). These short-term measurement techniques

include automated head chambers (GreenFeed, C-Lock Inc., Rapid City, USA; Zimmerman and Zimmerman, 2012), the CH_4:CO_2 ratio method (Madsen et al., 2010), the sniffer technique (Garnsworthy et al., 2012) and the handheld laser methane detector (LMD; Chagunda et al., 2009). Concerns regarding the use of each of the short-term measurement techniques relate to accuracy, sensitivity, repeatability and precision of data obtained (Hammond et al., 2016).

Researchers in South Africa (Grobler et al., 2014) have recently used the LMD as short-term measurement tool to estimate CH_4 emissions rate or production from individual ruminants under grazing conditions due to its convenience, practicality and cost-effectiveness. The LMD is a portable device to repeatedly measure CH_4 concentration (ppmv.m) from the air plume or column out to 3 m from the animal's nostrils or mouth during short periods of time. The resulting data consist of a series of small and large peaks which represent the animal's respired and eructated CH_4, respectively (Ricci et al., 2014). The measured CH_4 concentration (ppmv.m) can then be scaled up to CH_4 production (g/d) by adjustment factors used by Chagunda et al. (2009). Hammond et al. (2016) summarised that positive, but reasonably weak relationships between CH_4 concentrations and CH_4 emissions measured in respiration chambers have been reported by Chagunda et al. (2013) ($n = 2$; $r^2 = 0.22$; $P<0.001$) and Ricci et al. (2014) ($n = 67$; $r^2 = 0.28$; $P<0.001$). To date, there have been no reports of comparisons between the LMD and the SF_6 techniques, and the majority of the few previous LMD studies were performed with animals in confinement; therefore justifying the need for a LMD and SF_6 technique comparison study under grazing conditions. In addition, a clear LMD protocol for measurement of CH_4 concentration from ruminants under grazing conditions is needed.

Thus, the aim of this study was to compare the SF_6 technique as described by O'Neill et al. (2011) with one implementation of the LMD technique using a modification of the operating protocol and calibration equations reported by Chagunda et al. (2009), for measuring enteric CH_4 production from lactating dairy cows grazing pasture. We hypothesised that CH_4 production data derived from the LMD technique using a modification of the operating protocol and calibration equations would be comparable with CH_4 production data derived from the SF_6 technique. Results from this study will be used

to improve the operating protocol and calibration equations of the LMD technique for use in grazing systems.

7.3 MATERIALS AND METHODS

This study was performed at the Outeniqua Research Farm, George, Western Cape Province, South Africa (33°58´S, 22°25´E; altitude 210 m above sea level), during early spring. The area represents the typical coastal temperate, pasture-based dairy systems of South Africa with a long-term mean annual precipitation of 732 mm, spread throughout the year. The use of experimental animals and the experimental procedure were approved by the animal ethics committee of the University of Pretoria (project number: EC078-15).

Six multiparous, lactating Jersey cows were selected for the technique-comparison study. Cows were selected based on their temperament (approachability while resting and grazing) to facilitate the collection of LMD data. Pre-experimental production parameters of the experimental cows were 17.7 ± 1.54 kg of milk per day (mean ± SD), 156 ± 44.1 days in milk, 4.3 ± 1.36 lactations, 381 ± 25.6 kg of body weight and 2.2 ± 0.12 body condition score (scale 1 to 5; Wildman et al., 1982). An additional three lactating cows, similar to the experimental cows, formed part of the study as mobile background samplers of ambient concentrations of both SF_6 and CH_4. Cows were individually fed in the milking parlour with a conventional dairy concentrate at a rate of 5.4 kg of dry matter (DM) per day split equally over two milkings (0700 h and 1500 h). For the rest of the day, cows strip-grazed irrigated, perennial ryegrass pasture which was freshly offered twice daily after milking (approximately 12 kg of DM/cow per day). Fresh water was always available.

Enteric CH_4 emissions from individual cows where measured concurrently both with the LMD (Chagunda et al., 2009) and the SF_6 technique as described by O'Neill et al. (2011) for grazing dairy cows, over a period of six consecutive days.

For the SF_6 technique: empty permeation tubes (P&T Precision Engineering Ltd., Naas, Co. Kildare, Ireland) were filled in July 2016 with 3.0 ± 0.14 g of SF_6 (mean ± SD). The release rate of SF_6 was 5.1 ± 0.50 mg/d and ranged from 4.2 to 6.1 mg/d one week prior insertion in rumen *per os*. Permeation tubes were calibrated in a dry incubator

(Labcon Incubator Model FS1M8, Johannesburg, South Africa) set at 39.0°C for 4 wk and the permeation tubes were weighed (Sartorius BP210S, Sartorius AG, Göttingen, Germany; 0.0001 g accuracy) every third morning. The SF_6 release rate was calculated by linear regression of the permeation tube weights obtained during the calibration period and only permeation tubes meeting the criteria of $R^2 > 0.9993$ were used. Cylindrical, back-mounted polyvinyl chloride (PVC) gas-collection canisters of 1700 mL with an initial sampling rate of approximately 0.47 ml/min were used to continuously sample eructated gasses over a 24 h period. The given sampling rate allowed for the evacuated canister to fill to approximately 40% over a 24 h sampling period. Canisters were mounted on the back of the cows using the simple back-mounted harness of van Wyngaard et al. (2018). Canisters were flushed prior use. This involved five cycles of evacuating to 98 kPa vacuum, filling with ultra-high purity nitrogen gas (999.99 g/kg nitrogen) and evacuating again to 98 kPa vacuum. Initial sampling rate was obtained by restricting flow with a stainless-steel capillary tube (1/16" OD x 0.2" ID; YY-RES-21503; LECO Co., Saint Joseph, MI, USA) cut to 50 mm length and crimped using a table top vice-grip until the specified flow was attained.

Ambient (background) concentrations of CH_4 and SF_6 were sampled by using three additional cows equipped with the same harness and canister as those used by the experimental cows with the single alteration that the flow inlet was on the back of the animal, pointing down, and not above the nostrils. Background cows were kept in one group with the experimental cows during grazing and milking. Single daily estimates of background concentrations for both CH_4 and SF_6 were used for all experimental cows by averaging the three background concentrations from each cow on each day. Oil vacuum gauges (R3A63G14B; SA Gauge (Pty.) Ltd., Durban, RSA) were used to measure vacuum of evacuated canisters prior to daily connection and removal of sample canisters.

Gas samples were extracted and sub-sampled from the sample canisters into three 12 mL glass vials (Labco Exetainer, Labco Ltd., Ceredigion, UK) using a piston sub-sampler (National Institute of Water and Atmosphere (NIWA) Ltd., Auckland Central, NZ). Gas samples were analysed with NIWA's modified gas chromatograph (GC) equipped with a Gilson Sample Changer (Gilson, Inc., Middleton, WI, USA) to analyse pressurised air samples in Labco Exetainers. The GC was fitted with a flame-ionisation detector and an

electron-capture detector (Hewlett Packard Model 6890, Palo Alto, CA, USA) using each an Alltech Porapak-Q 80-100 mesh column (3.6 m × 3 mm stainless steel; Grace Davison Discovery Sciences, Deerfield, IL, USA) in parallel configuration. Three standards of SF_6 ("Lenny" 18.02 pptv, "Monty" 162.6 pptv, "Horatio" 969 pptv; NIWA) were associated with the analyses of each batch. In addition, the middle standard "Monty" is also a CH_4 standard (24.11 ppmv). Methane production (g/d) was calculated using equation 2 from the study of Williams et al. (2011).

The LMD (SA3C32A LaserMethane mini; Tokyo Gas Engineering Co. Ltd., Ota-ku, Tokyo, Japan) was set to take measurements every 0.5 s. Measurements were made once a day for a period of 4 min over six consecutive days in either the morning (05h-06h), mid-day (12h-13h) or late-afternoon (17h-18h) while the operator stood a maximum of 3 m from the cow's nostrils or mouth. This LMD operating protocol was based on experiment 2 from the study of Ricci et al. (2014) with the modification of repeating the measurement period over six and not three consecutive days and changing the time of day of measurement. This was done to reduce animal variation and to avoid the risk of bias associated with the diurnal biphasic pattern of CH_4 emissions exhibited by grazing ruminants (Hegarty, 2013). In the study of Ricci et al. (2014) feed was offered unrestricted once a day between 0800 h and 0900 h. A video recording was made of the display screen of the LMD during each sampling period for each cow. This was done to capture the data flashed every 0.5 s. This protocol allowed for a total of 480 spot samples per cow/d, similar to that reported by Ricci et al. (2014). All measurements were taken while the cows were on pasture. Animal position at time of measurement was recorded (lying or standing). The measured LMD concentrations were not adjusted for distance as the plume density of cow's breath is unknown and assumed to be one (Chagunda, personal communication, 2017). No offset values for ambient CH_4 concentrations were set due to technical issues. Raw LMD data (ppmv.m) were converted to CH_4 production (g/d) using the following equation (Chagunda, personal communication, 2017); adapted from Chagunda et al., 2009):

$$Y = d \times (5.76 \times m) \quad (1)$$

Where, Y = CH_4 production (g/d), d = 0.31 (animal lying down) or 0.38 (animal standing), and m = average methane concentration (ppmv.m).

The converted LMD data for each measurement were plotted on a graph and the standard deviation (SD) x1 of the data set was used to set a threshold between respired and eructated CH_4 (Chagunda, 2013). Thereafter, only the small peaks reflecting the increase in CH_4 production for both exhalation and eructation were used for analysis (Ricci et al., 2014).

The four min LMD measuring protocol was rarely achieved during this grazing study. This was mainly due to the effects of ambient conditions (humidity, precipitation, wind speed and direct sunlight) and animal grazing behaviour (moving head rapidly from side to side and walking while grazing) making the practicality of the technique problematic.

The modified Z-score was used to identify outlying data obtained from both techniques. Data associated with 'modified Z-scores' of >3.5 (absolute value) were labelled as outliers (Berndt et al., 2014). Some outlier data from the SF_6 technique were excluded as a result of leaking canisters that overestimated CH_4 production. Descriptive statistics such as mean, median, maximum, minimum, SD and CV values were calculated to determine the distribution of the data. The residuals were acceptably normal with homogeneous treatment variances. A linear regression analysis was done on both techniques with CH_4 production as the response variate and days as the constant. Methane production from this study was analysed as a two-sample t-test with analysis of variance (ANOVA) to test for differences between the two techniques. Treatment means were compared using Tukey's least significant difference test at the 5% level of significance (Snedecor and Cochran, 1980). Treatment mean trends were specified at a significance level of $0.10<P<0.05$. Data were analysed using the statistical program GenStat® (Payne, 2014).

7.4 RESULTS AND DISCUSSION

A typical 4 min dataset from a randomly selected cow showing estimated CH_4 concentrations and predicted CH_4 productions obtained by the LMD technique is shown in Figure 7-1. The threshold between respired (below) and eructated (above) CH_4 is indicated

by the horizontal dotted line which represents SDx1 of the given dataset. The study of Ricci et al. (2014) reported a more robust 2-step process to differentiate between these two levels of CH_4 concentrations to increase the sensitivity of the technique to detect a difference.

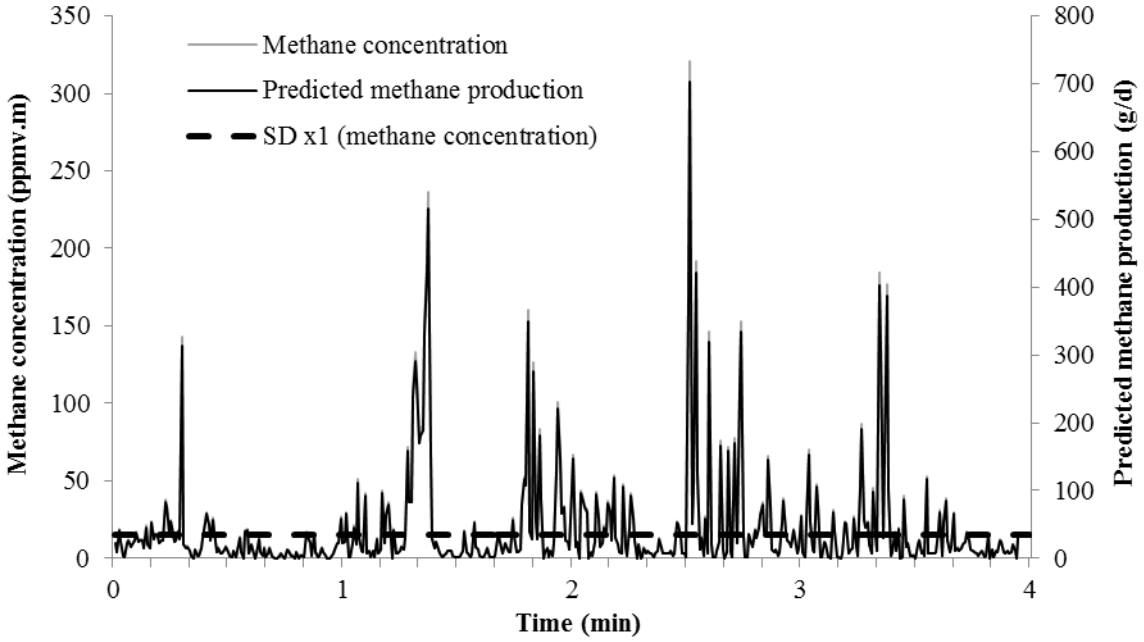

Figure 7-1 A typical 4 min dataset from a randomly selected cow indicating methane (CH_4) concentration (ppmv.m) and predicted CH_4 production (g/d) as determined by the laser methane detector (LMD) showing the small and large peaks. The horizontal dotted line represents the standard deviation x1, which indicates a threshold between respired (below the line) and eructed (above the line) methane.

However, this process is laborious and complicated to apply in practice and can be seen as a disadvantage of the LMD to be used as a rapid and simple monitoring technique (Ricci et al., 2014). Furthermore, this process was not applied because testing for treatment effects was absent from this study.

Table 7-1 presents the mean CH_4 concentration (ppmv.m) from the LMD, the calculated CH_4 production data (g/d) as well as the maximum respired and eructated CH_4 production data. The difference between respired and eructated CH_4 in the current LMD operating protocol does not present any advantages to determining absolute CH_4 production, because the typical unit for CH_4 production is a mass or volume (g or L) per unit of time (min, h, or d), such as g/d, which includes the combination of respired and eructated CH_4.

Table 7-1 Mean ± standard deviation of spot samples per 4 min sampling period, methane (CH_4) concentration (ppmv.m) measured by the laser methane detector (LMD) and calculated CH_4 production data (g/d) as well as maximum respired and eructated CH_4 production data from lactating Jersey cows grazing perennial ryegrass pasture.

Item	
Spot samples per sampling period	165 ± 61.6
CH_4 concentration measured by LMD (ppm_v.m)	35.7 ± 7.11
Calculated CH_4 production data (g/d)[1]	82.6 ± 49.79
Maximum respired CH_4 production data (g/d)	77.5 ± 16.95
Maximum eructated CH_4 production data (g/d)	586 ± 104.4

[1] Methane production (g/d) was calculated using equation 1 (Chagunda, personal communication, 2017); adapted from Chagunda et al., 2009).

However, Ricci et al. (2014) reported that this separation of CH_4 improved the ability of output data obtained from the LMD technique to show contrast between dietary treatments. Nonetheless, there are other advantages in separating respiration and eructation events, such as being able to obtain an indication of the amount of time spent ruminating. This could be used e.g. as a mechanism to examine daily intake (Metz, 1975) and to obtain an index of feed quality in terms of cell wall constituents (Welch and Smith, 1969) and physical effective neutral detergent fibre (Mertens, 1997).

Furthermore in Table 7-1, the number of spot samples per 4 min sampling period represents only 34% of the possible 480 spot samples as allowed by the specified LMD operating protocol. This was mainly a result of the difficulty to maintain the laser of the LMD on the nostrils or mouth of the cows on pasture due to a combination of factors such as ambient conditions and head movement of the cows while grazing. The reason for the different time frames of measurement each day (morning, mid-day or late-afternoon) was to accommodate the varying ambient conditions between milking, such as high relative humidity (common in coastal regions), precipitation (temperate rainfall region), wind speed, which all affect CH_4 concentration (Teeranavattanakul, 2010) and direct sunlight that impairs the visibility of the laser.

The mean CH_4 concentration (35.7 ppmv.m) as measured by the LMD in this study is similar to that (36.6 ppmv.m) measured 3 m from the nostrils of four Jersey heifers fed a total mixed ration (Grobler et al., 2014). Furthermore, when compared with the current study, Ricci et al. (2014) reported a slightly higher average CH_4 concentration (53.5 *vs*. 35.7 ppmv.m) measured 1 m from the nostrils of 72 steers (body weight of 673 kg) fed two total mixed ration diets with different forage-to-concentrate ratios. The number of spot samples per animal varied from study to study with 480 spot samples used by Grobler et al. (2014), 1440 by Ricci et al. (2014) and 2880 collected in the current study (of which only approximately 980 were obtained). This suggests that the LMD operating protocol followed in the current study did not bias the LMD output data by obtaining insufficient spot samples. Other LMD studies (Chagunda et al., 2009, 2013; Chagunda and Yan, 2011) reported higher CH_4 concentrations for dairy cows: 326 ppmv.m for lactating and 204 ppmv.m for dry cows (n = 110; measured 3 m from the animal's nostrils); 396 ppmv.m for dry cows (n = 2; measured 2.75 m from the animal's nostrils); and 417 ppmv.m for dry and lactating cows (n = 8; measured 2.3 m from the animal's nostrils); respectively. Reasons for the relative large variation in LMD results between studies are not clear. Perhaps the assumptions made by the LMD technique that animal-to-animal plume morphology is constant (Chagunda et al., 2009) is inaccurate and could, therefore, account for the discrepancy in results from this study and previous studies. In addition, whether distance corrections in estimated CH_4 concentration should be made or not is unclear, because the plume density of cow's breath is unknown and assumed to be one (Chagunda et al., 2009;

Chagunda, personal communication, 2017). In the current study, if corrections were made for measurement distance from the cow's nostrils, the estimated CH_4 concentration and predicted CH_4 production derived from the LMD technique would be 3-fold lower which will cause a larger discrepancy in results between this study and previous studies. This emphasises the need to develop a standardised measuring and data interpretation protocol for the LMD.

Descriptive statistics for both the LMD and the SF_6 technique are presented in Table 7-2. The CV of the SF_6 data in the current study is higher than that of previous studies. The study of Deighton et al. (2014) reported that formerly published between-animal CV determined using the SF_6 technique ranged from 11 to 24.5%. However, it should be highlighted that CH_4 emissions measured during these studies were from animals in confinement and not under grazing conditions. Grazing studies are renowned for the challenges in CH_4 emission measurements as seen here.

Table 7-2 Descriptive statistics (number of samples (n), mean, standard deviation (SD), median, minimum, maximum, skewness, kurtosis and coefficient of variation (CV)) for methane production (g/d) measured by the laser methane detector (LMD) and the sulphur hexafluoride tracer gas (SF_6) technique.

Method	n	Mean	SD	Median	Minimum	Maximum	Skewness	Kurtosis	CV
LMD	29	82.6	49.8	75.3	15.5	205	0.865	0.044	60
SF_6	22	348	143	309	171	730	1.16	0.741	41

In this study, the high between-cow CV (0.41) of the SF_6 technique data was a result of the adjusted flow rate to fill only 40% of the sample canister instead of the traditional 50% (Johnson et al., 2007) over a 24 h sampling period. The reasoning behind the adjustment was an attempt to reduce error owing to the non-linear flow exhibited by capillary tubes (Deighton et al., 2014). In some instances, this adjustment resulted in a reduced amount of gas sampled over a 24 h period, affecting the piston sub-sampler's ability to extract sufficient sample from the canisters. This is problematic especially when SF_6 concentration is analysed in parts per trillion. Therefore, an insufficient gas sample led to low SF_6 concentration values and ultimately overestimating CH_4 production. Extreme

overestimates over the first two days of measurement were identified as outlying data and discarded. The flow rate was corrected after the second day of measurement to ensure a 50% canister fill rate over 24 h.

The high CH_4 production values obtained using the SF_6 technique over the first two days of measurement is evident in Figure 7-2, where after it stabilised post the flow rate adjustment. As result, there is an indication (P=0.094) of a linear trend over five days with the SF_6 technique, but no indication (P=0.911) of a trend over five days with the LMD technique as evident in Figure 7-2, making the LMD superior in repeatability and precision over the SF_6 technique in the current study.

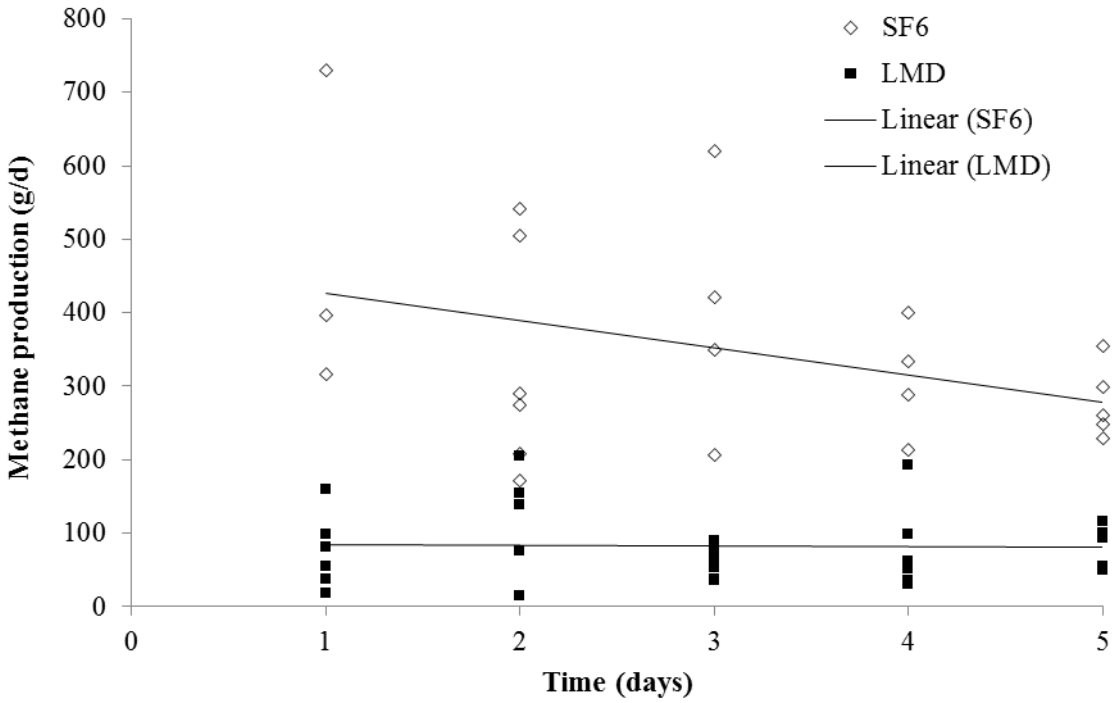

Figure 7-2 Fitted and observed relationship between methane (CH4) production as obtained by using the laser methane detector (LMD) and the sulphur hexafluoride tracer gas (SF6) technique over five consecutive days.

Despite the high between-cow CV, the CH_4 production of 348 g/d as measured by the SF_6 technique from Jersey cows with an estimated DM intake of 16.5 kg of DM/d (small breed cow producing 20 kg of milk/d with a milk fat and protein content of 4.5%

and 3.5%, respectively; NRC, 2001) fits the universal linear predictive regression of Charmley et al. (2016). This indicates that CH_4 production as measured by the SF_6 technique is biologically valid, whereas the CH_4 production as measured by the LMD is an extreme underestimate given the specified animal parameters when compared with the literature (du Toit et al., 2013; Charmley et al., 2016).

The high between-cow CV (60%) of the LMD data reflects the difficulty of using the LMD under grazing conditions which can, in part, be ascribed to the different time frames of measurement each day. Furthermore, the high between-cow CV can also be partly ascribed by the (1) high resolution and fast response time of the LMD to small fluctuations in CH_4 concentrations during measurement (Ricci et al., 2014), and (2) the current LMD device accuracy of only ±10% as noted in the device manual. These factors will most certainly affect the accuracy of the estimated CH_4 concentrations derived from the LMD technique and will have a roll-over-effect when scaled up to CH_4 production which could result in under- or over-estimates of 10%. Therefore, it is suggested that the accuracy of the LMD device needs to be improved before it can be considered as short-term measurement tool to estimate enteric CH_4 concentrations from ruminants.

The lack of setting offset values for ambient CH_4 concentrations in the LMD measures is a shortcoming of this study; however we believe that it will not be a limiting factor affecting the results (in terms of CV) of the LMD technique, because cows grazed in close proximity of each other during measurement, hence the ambient concentrations would have been similar between cows. Lower between-cow CV as reported from previous LMD studies are mainly due to experiments conducted indoors under more controlled environments and where animals were housed individually and restrained during measurement (Chagunda and Yan, 2011; Chagunda et al., 2013; Ricci et al., 2014).

The measured and predicted CH_4 production data from the six cows over five consecutive days derived from the SF_6 and LMD technique, respectively, have a weak linear relationship (r^2 = 0.19; P=0.466). This indicates the inaccuracy of the LMD technique with modified operating protocol and calibration equations compared with the SF_6 technique of O'Neill et al. (2011). Unsurprisingly, the two-sample t-test gave very strong evidence (P<0.001) that with the SF_6 technique mean CH_4 production (348 g/d) was significantly higher compared with the mean CH_4 production measured with the LMD

technique (82.6 g/d) as shown in Table 7-3. This suggests that the LMD technique with modified operating protocol and calibration equations underestimates CH_4 production in the current study by as much as 76%.

Table 7-3 Mean methane production as measured using the sulphur hexafluoride tracer gas (SF_6) technique and the laser methane detector (LMD) from Jersey cows grazing perennial ryegrass pasture.

Item	Methane measurement technique		SED[1]	P-value
	SF_6	LMD		
Methane production (g/d)	348.2	82.6	31.84	<0.001

[1] SED–standard error of difference.

In a review of short-term emission measurements it was concluded that raw short-term emissions data, such as LMD data, can be useful for screening animals for selective animal breeding and ranking purposes but not necessarily for CH_4 production, mainly due to substantial assumptions made on the homogeneity of animal behaviour and physiology (Hegarty, 2013). However, the LMD results of this study suggest that sources of variation caused by factors such as animal movement or micrometeorology should be intensively quantified and investigated, before recommending the LMD technique for outdoor CH_4 monitoring. Therefore, the current measuring protocol of the LMD technique and the LMD device used in this study needs to be reconsidered for use in monitoring CH_4 concentration in grazing systems.

In an attempt to improve the relationship between daily CH_4 emissions as measured by the SF_6 technique, and the CH_4 concentration as measured by the LMD technique, data from d 1 and 2 were excluded due to the unreliable data from the SF_6 technique because of small sample sizes. Data from d 4 was also excluded as the data was inconsistent. Data was also transformed, but did not reduce the variation. Linear regression analysis was used to establish if there is a relationship between methane emissions measured by the two techniques, SF_6 technique (Y-variate) and LMD technique (X-variate). The following regression equation was derived from the data:

$$Y = (LMD \times 2.1) + 203.8 \quad (2)$$

Where, Y = CH_4 production (g/d), LMD = CH4 concentration derived from the LMD technique (ppmv.m).

The linear relationship was not significant (P>0.05) with an adjusted R^2 value of only 18%, thus not reliable, but there is an indication of a positive linear trend between the CH_4 emissions of the two techniques. To create a more reliable prediction equation more animal numbers and a SF_6 dataset with lower between-cow CV will be required.

It has been established that DMI is the main driver for CH_4 production, followed by other factors such as dietary neutral detergent (NDF) content (Niu et al., 2018). In grazing systems, pasture DMI is affected by several animal, plant and grazing management factors, such as rumen fill (Boudon et al., 2009), pasture quality (such as NDF content influenced by, inter alia, plant growth stage and plant species) and pasture allowance (Bargo et al., 2003), respectively. Therefore, further comparisons with an improved experimental design with larger animal numbers, with different pasture allowances (low, medium and high) and different pasture species or quality is needed to develop a robust LMD technique protocol for grazing systems.

7.5 CONCLUSIONS

Methane concentration (ppmv.m) as measured by the LMD from grazing dairy cows was scaled up to CH_4 production (g/d) and compared with CH_4 production results from the same grazing dairy cows as measured with the SF_6 technique. Results indicate that the LMD underestimated CH_4 production of lactating Jersey cows grazing perennial ryegrass pasture by 76% when compared with CH_4 production as measured by the SF_6 technique. However, results should be interpreted with caution as the number of animals with sufficient data points might be of concern. Additionally, the LMD operating protocol used in this study exhibited two shortcomings, 1) measurements were taken at different times of the day, and 2) no offset values for ambient CH_4 concentrations were set. Furthermore, observations from this study confirm that CH_4 concentrations measured by the LMD using the ‘conventional’ operating protocol are exceedingly sensitive to certain ambient

conditions affecting the output data. Findings of this study indicate that there is a need to improve the LMD operating protocol and scale-up factors to accurately convert CH_4 concentration (ppmv.m) to CH_4 production (g/d).

7.6 ACKNOWLEDGEMENTS

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CHAPTER 8

General discussion

8.1 CONCENTRATE SUPPLEMENTATION

The effect of concentrate supplementation on methane (CH_4) emissions from confined Holsteins and productivity of Holstein cows on ryegrass-dominant pasture is well known, but not from Jersey cows especially when grazing tropical pasture. This research may constitute the first research in which enteric CH_4 emissions from grazing Jersey dairy cows has been measured and reported on.

Expectedly, it was found that concentrate supplementation increased total dry matter intake (DMI) and milk yield, and decreased pasture DMI on both ryegrass-dominant and kikuyu-dominant pasture systems. Methane mitigation efficacy of concentrate supplementation was more prominent in late-summer when kikuyu is the dominant pasture. Kikuyu has inherently higher fibre content than perennial ryegrass, and the fermentation of fibre increases CH_4 emissions, hence providing more opportunity to reduce CH_4 emissions in kikuyu-based pasture systems. In this pasture system, a high level of concentrate supplementation increased enteric CH_4 production by 17% but reduced CH_4 yield (g/kg of DMI) by 14% while CH_4 intensity (g/kg of milk yield or energy-corrected milk yield [ECM]) was reduced by approximately 40%. On perennial ryegrass-dominant pasture, only CH_4 intensity (g/kg of milk yield) was reduced by 20% when supplementing concentrate. According to Knapp et al. (2014), a maximum reduction of 15% in CH_4/ECM can be achieved by increased concentrate feeding. This research has shown that a much higher reduction in CH_4/ECM is achievable when supplementing increased levels of concentrate, especially on tropical pastures.

Results from the rumen studies supported the milk response to some extent, but did not really help to explain the CH_4 results. An increased propionate concentration was expected as concentrate feeding level increased from 0 to 8 kg, especially where the concentrate to pasture intake ratio was close to 1:1, but this did not transpire. It is very difficult to defend these volatile fatty acid (VFA) results as it does not represent the obvious outcome. However, in the review study of Bargo et al. (2003), it was evident that

VFA concentration response to concentrate supplementation was highly inconsistent. This suggests that there is not a simple relationship between concentrate supplementation and VFA concentration. To complicate even further, the implemented strict daily herbage allowance could have caused competitive and aggressive behaviour between cows and some cows may have had variable pasture DMI from day to day. This could lead to relative high between-cow coefficients of variation for VFA concentrations, hence the lack of treatment response.

In conclusion, this research has provided an understanding of the potential use of concentrate supplementation as CH_4 mitigation strategy for dairy cows in pasture-based systems. The findings of this research may have application with respect to improving the accuracy of the South African National Greenhouse Gas (GHG) Inventory. Furthermore, it can also be useful for upcoming meta-analysis studies evaluating the effect of diet on enteric CH_4 emissions in improving existing enteric CH_4 prediction equations. Finally, the impact that concentrate supplementation could have on the total carbon footprint, on- and off-farm, as well as the effect on profitability at the farm scale should not be overlooked.

8.2 NATIONAL GREENHOUSE GAS INVENTORY

The current livestock sector of the South African National GHG Inventory is based on tier 2 methodologies, in accordance with the IPCC (2006) good practice guidelines. One of the aims of this research was to compare directly measure enteric CH_4 emissions from grazing dairy cows with that of the calculated values used by the current national GHG inventory. This comparison was done for both kikuyu-dominant and ryegrass-dominant pastures (Table 8.1). Total daily CH_4 production of grazing dairy cows was calculated using Eq. 1 to 5 of du Toit et al. (2013). Actual values rather than national herd averages were used, where applicable. These values were obtained from Chapter 2 (kikuyu-dominant pasture) and Chapter 3 (ryegrass-dominant pasture). Actual body weight, milk yield and dry matter digestibility (DMD) values were used for Eq. 1 (DMI); actual body weight and DMI values were used for Eq. 3 (intake needed relative to maintenance); actual DMD values were used for Eq. 4 (Ym); and actual gross energy intake values were used for Eq. 5 (CH_4 production).

Table 8-1 Comparisons between directly measured and calculated dry matter intake, methane energy per gross energy intake and total daily methane production of Jersey cows fed different levels of concentrate, and grazing either kikuyu-dominant or ryegrass-dominant pastures**.**

Item[1]	DMI (kg/d)		Difference (%)	Y*m* (%)		Difference (%)	CH_4 production (g/d)		Difference (%)
	Calculated	Measured		Calculated	Measured		Calculated	Measured	
Kikuyu-dominant pasture									
Concentrate level[2] (kg)									
0	11.7	11.2	4.6	5.36	8.91	-66	196	323	-65
4	13.5	12.8	5.2	5.95	8.97	-51	246	367	-49
8	14.8	15.6	-5.6	6.87	7.85	-14	342	378	-10
Ryegrass-dominant pasture									
Concentrate level (kg)									
0	12.2	13.4	-10	5.94	6.38	-7	257	258	0
4	13.5	16.4	-22	7.09	6.12	14	372	321	14
8	14.3	18.0	-26	7.72	5.30	31	442	302	32

[1] DMI–dry matter intake; Y*m*–methane energy per gross energy intake; CH_4–methane.
[2] As fed basis.

It is evident in Table 8.1 that the prediction equation used for DMI (Eq. 1) is a good estimate for cows grazing kikuyu-dominant pasture, but not for cows grazing ryegrass-dominant pasture. This suggests that Eq. 1 in du Toit et al. (2013) is not sensitive to changes in feed DMD. It is well known that DMI is positively correlated with feed DMD, and that pasture substitution is negatively correlated with pasture DMD.

In general, Y*m* reduces when the starch:fibre ratio of ruminant diets is increased (Beauchemin et al., 2008). Remarkably, the calculated Y*m* values in Table 8.1 show the inverse as concentrate feeding level increases from 0 to 8 kg/d. This implies that Eq. 4 in du Toit et al. (2013) does not account for changes in the starch:fibre ratio of ruminant diets. Enteric CH_4 emissions from tropical or sub-tropical pastures, such as kikuyu, cannot be categorised with that of temperate pastures, such as ryegrass, as evident in the measured Y*m* values in Table 8.1. Therefore, the default Y*m* value of 6.5% (IPCC, 2006) is not representative of ruminants grazing tropical pastures. Furthermore, it is evident that the calculated Y*m* values underestimate measured values on kikuyu-dominant pastures and overestimate measured values on ryegrass-dominant pasture. The trend in CH_4 production comparison values corresponds to that of the trend of the Y*m* comparison values. In Table 8.1, measured CH_4 production, averaged across concentrate feeding level, is underestimated by 42% and overestimated by 15% for cows grazing kikuyu-dominant pasture and ryegrass-dominant pasture, respectively. Combined, measured CH_4 production is underestimated by 13%.

The findings of this research signify that the tier 2 methodologies used to build the current livestock sector of the South African National GHG Inventory is not sensitive to changes in DMD and starch:fibre ratio of ruminant diets. It is suggested that the current South African National GHG Inventory need to be updated with tier 3 results or results from improved tier 2 methodologies.

8.3 DIETARY NITRATE SUPPLEMENTATION

Dietary nitrate fed to grazing dairy cows showed some promise as CH_4 mitigation strategy. Dietary nitrate addition (up to 23 g of dietary nitrate/kg of DM) tended to linearly reduce daily CH_4 production, CH_4 yield and GE lost as CH_4 energy (Y*m*) of cows grazing

kikuyu-dominant pasture by as much as 11, 14, and 15%, respectively. However, cows partially refused concentrate containing 23 g of dietary nitrate/kg of DM, which led to a 12% decrease in milk yield on kikuyu-dominant pasture. It was believed that the partial refusal of concentrate was manifested by the organoleptic properties of dietary nitrate. Despite this, rumen fermentation was not adversely affected. Therefore, surprisingly, when dietary nitrate was fed at a level of 15.2 g/kg of DM to cows grazing perennial ryegrass-dominant pasture a 5% increase in milk fat content was evident, which could contribute to increasing the farmer's milk cheque. The potential toxicity factor of nitrate along with fluctuating nitrate levels in the basal diet, imposed by several environmental and anthropogenic factors, makes it currently not a viable option as CH_4 mitigation strategy in pasture-based systems.

8.4 DISCREPANCY IN METHANE EMISSIONS

The quantitative differences in CH_4 production (g/d) among chapters can be ascribed to several factors of which all can influence enteric CH_4 emissions at varying levels. These factors include parity, days in milk, breed, heat stress and pasture botanical composition and quality to name a few. A more acceptable unit of measure is CH_4 yield (g/kg DMI) and CH_4 intensity (g/kg of ECM or milk yield) and is mainly driven by DMI and milk response, respectively. In a global meta-analysis study of Niu et al. (2018), it has been established that DMI is the main driver for CH_4 production, followed by other factors such as dietary neutral detergent fibre (NDF) content. Thus, the discrepancy in CH_4 yield among chapters can be described, in part, by the difference in pasture DMD, imposed by the NDF content of the pasture. Accordingly, CH_4 yield will be expected to be higher on kikuyu-dominant pasture than ryegrass-dominant pasture, because kikuyu pasture has an inherently higher NDF content.

Another explanation for the discrepancy in CH_4 production among chapters can be described, in part, by the organic matter intake (OMI) and not so much the DMI. This is because ash content in ruminant diets does not contribute to CH_4 production. The average DMI for chapter 2 (kikuyu) and chapter 3 (ryegrass) is 13 and 16 kg/d, respectively. However, when adjusting for ash content the OMI for chapter 2 (kikuyu) and chapter 3

(ryegrass) is 12 and 14 kg/d, respectively. When OMI increases, rumen passage rate increases. In return, an increased passage rate will result in less time spent by microbes to ferment feed and, theoretically, less CH_4 will be released. Thus, also partly explaining the difference in average CH_4 production among chapter 2 and 3 (356 *vs.* 294 g/d, respectively).

8.5 BACKMOUNTED HARNESS AND TECHNIQUE COMPARISON

This research has provided a novel, low-cost back-mounted harness for grazing dairy cows in facilitating the SF_6 technique in enteric CH_4 measurement while focusing on animal welfare. This simplified harness may also have application as a mount for a wide range of electronic sensors that are increasingly being used in research on grazing dairy cows.

A component of this research, focussed on the comparison of two CH_4 measurement techniques, the sulphur hexafluoride tracer gas (SF_6) technique and the laser methane detector (LMD) technique. Methane concentration (ppmv.m) as measured by the LMD technique from grazing dairy cows was scaled up to CH_4 production (g/d) and compared with CH_4 production results from the same grazing dairy cows as measured with the SF_6 technique. There were several shortcomings in this study, but it can be concluded with confidence that the current LMD technique is not ready for outdoor use or currently capable to accurately estimate CH_4 production of livestock for inventory purposes.

8.6 FUTURE WORK IN THIS AREA

- A full GHG life cycle assessment on the South African pasture-based dairy sector is encouraged.
- More research is needed to fully elucidate the role of rumen fermentation parameters as proxies for enteric CH_4 emissions in grazing dairy cows.
- The potential of dietary nitrate as CH_4 mitigation strategy for grazing ruminants need further investigation.

- Future research is encouraged to evaluate the effect of nitrate supplementation on milk fat content.
- Future research is encouraged to evaluate the inclusion of feed flavourants to possibly overcome the organoleptic properties of dietary nitrate.
- Findings of this study indicate that there is a need to improve the LMD operating protocol and scale-up factors to accurately convert CH_4 concentration to CH_4 production.

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CHAPTER 9

Conclusion

Enteric methane (CH_4) emissions were successfully measured from lactating Jersey cows grazing kikuyu-dominant pasture in late-summer and perennial ryegrass-dominant pasture in early-spring. Concentrate supplementation showed to be an effective enteric CH_4 mitigation strategy for grazing, lactating dairy cows. This strategy was more effective for cows grazing kikuyu-dominant pasture where CH_4 emissions per kilogram milk were reduced by 40% compared with 20% for cows grazing ryegrass-dominant pasture. The addition of dietary nitrate as enteric CH_4 mitigation strategy for grazing dairy cows was not as successful. Enteric CH_4 emissions only tended to reduce when dietary nitrate was fed to dairy cows grazing kikuyu-dominant pasture. From this array of grazing studies, a novel back-mounted harness was developed for grazing dairy cows, with the focus on animal welfare. When comparing the directly measured enteric CH_4 emissions with the calculated CH_4 emissions of the South African National Greenhouse Gas Inventory, the general perspective is that the current inventory values are underestimating directly measured CH_4 emissions from grazing dairy cows. Finally, a component of this research has shown that there is a need to improve the operating protocol and scale-up factors of the laser methane detector technique before it can be used for inventory purposes.

ADDENDUM A

SF_6 technique: Standard operating protocol

A.1 INTRODUCTION

This section will provide for a standard operating protocol for the sulphur hexafluoride tracer gas (SF_6) technique for grazing dairy cows as was developed from and implemented by the studies in this dissertation.

A.2 TIME FRAME

When planning to implement this technique a margin of at least six months are required for the initial sourcing, fabrication and preparation of equipment. Thereafter, two months are sufficient for equipment preparation prior the onset of a new SF_6 trial.

A.3 PERMEATION TUBE

Design

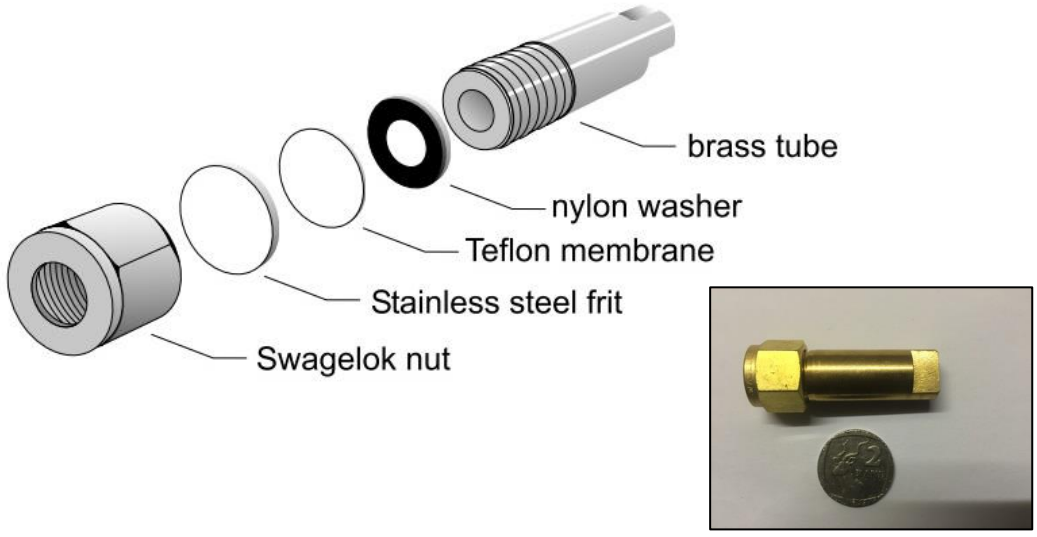

Figure A-1: Breakdown of the permeation tube components (diagram from Lassey et al. (2001)). Photo insert: size comparison of permeation tube with a South African two Rand coin.

Table A-1 Complete component description of the permeation tube

Components	**Description**
Brass tube (body)	Drilled brass rod: 14.3 mm OD; 45 mm length; 8 mm ID; 37 mm depth of cavity. Tapered and threaded at tip to fit Swagelok nut and fluted at base to anchor tube while torqueing (prepared by P&T Precision Engineering Ltd., Kildare, Ireland; and Kriess Hydraulics CC, George, South Africa).
Nylon washer	1 mm thickness; 11 mm OD; 5.3 mm ID (source: P&T Precision Engineering Ltd., Kildare, Ireland).
Teflon membrane	Polytetrafluoroethylene (PTFE): 0.2 mm thickness; 12.7 mm OD (prepared by P&T Precision Engineering Ltd., Kildare, Ireland).
Stainless steel frit	Sintered 316 stainless steel frit: 1.6 mm thickness; 12.7 mm OD (prepared by P&T Precision Engineering Ltd., Kildare, Ireland).
Swagelok nut	Brass Swagelok nut with 9.5 mm ID window (part# B-602-1; Swagelok®, Ohio, USA).

Pre-filling preparation

Allow at least 5 wk to fill, assemble and calibrate permeation tubes. Prior filling, bodies and nuts should be submerged and 'washed' in acetone for 24 h to remove any oily residues from the manufacturing process. From here on permeation tube components should be handled with pliers or gloves to avoid the transfer of oily residues. Following the acetone wash, tube components should be dried at 100°C for 12 h and allowed to cool down to ambient temperature before commencing the SF_6 filling process.

Pairing of bodies and nut components

Following pre-filling preparation each brass body is paired with a nut component (nut, stainless steel frit, Teflon membrane and nylon washer – the layering sequence is very important) to form a permeation tube of which weight is recorded using a scale with 0.0001 g accuracy (Sartorius, Göttingen, Germany). This pair should stay unchanged throughout the pre-weighing and filling of tubes.

Filling

Avoid cross contamination of SF_6: it is critical to fill, calibrate and store permeation tubes at least 10 km from sites where canisters and sampling lines are assembled and stored, and where breath samples are extracted from canisters and analysed.

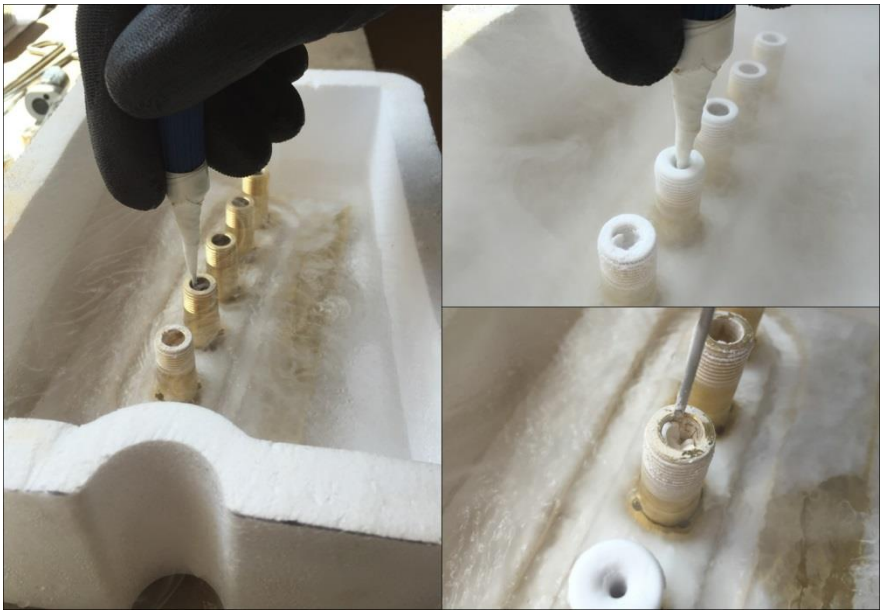

Figure A-2 Filling permeation tubes with SF_6 gas using a needle connected to a regulated gas cylinder via a flexible gas hose. Permeation tubes are secured in a machined brass plate submerged 3/4 in liquid nitrogen in a polystyrene container.

Secure five brass bodies in a flat brass block that reside in a polystyrene container (filling station; Figure A-2). Fill the container with liquid nitrogen up to the tread of the brass bodies. Avoid liquid nitrogen from entering the brass bodies. Allow 1 min for the brass bodies to reach liquid nitrogen temperature. The SF_6 gas is transferred from the SF_6 cylinder via a gas hose attached with an 18 gauge needle on the open-end. The needle is halfway inserted in the brass body and flow is continuously regulated with a scientific regulator (Afrox, Johannesburg, South Africa) attached to the SF_6 cylinder to avoid excess flow of SF_6 out of the brass body and at the same time avoiding solidification of SF_6 in the needle.

Brass bodies are filled one at a time up to the level where the tread begins (this should result in a fill of approximately 3 g of SF_6). Once all five brass bodies are filled, SF_6 sediment is carefully removed from the rim towards the cavity of the brass body using two needles (a sharp blade could also be used). ***When clearing the brass body rim of SF_6 sediment avoid damaging the surface as this could impede the sealing ability of the nylon washer and could also result in end-weight discrepancies.*** A needle is used to compact the SF_6 sediment in the brass body cavity. In some cases after compaction, more SF_6 is needed to achieve the specified filling level.

After filling and removal of rim sediment, the paired nut component (with the correct sequence of components) is carefully placed on the brass body, one at a time, and finger tightened. A torque wrench is used to torque the nut component to 5 N.m where after the permeation tube is removed from the filling station and left to thaw at ambient temperature. Once thawed, permeation tubes are re-torqued to 5 N.m to ensure a good seal and placed as one batch in a dry incubator (Labcon, Johannesburg, South Africa) set at 39.0°C.

Calibration of release rate

Permeation tubes are weighed (Sartorius, Göttingen, Germany; 0.0001 g accuracy) every third day at the same time for four to five weeks while stored in the incubator set at 39.0°C to obtain a nine to eleven point regression curve. Only permeation tubes with a R^2 > 0.999 are used for animal trials. ***Therefore, it is important to initially fill 50% more permeation tubes than needed for the trial.*** The release rate will be in the range of 4 to 7 mg of SF_6/d. ***When selecting the trial permeation tubes from the passed calibrated set, it is important to select permeation tubes with a narrow release rate range to restrict carry-over variation.***

A.4 CANISTER

Design

To attain a cylindrical canister with a volume of 1 700 mL, polyvinyl chloride (PVC) pipe (90 mm OD x 2.7 mm wall thickness; pressure class 6; Suid-Kaap Besproeing, George, South Africa) is cut in 300 mm lengths and closed off with PVC end-caps (90 mm ID; Suid-Kaap Besproeing) using PVC contact adhesive. Sandpaper is used to rough the surface for a better bond before end-cap attachment. Allow PVC contact adhesive to dry completely.

Figure A-3 Complete canister with quick release valve.

Canister valve consist of a brass male elbow fitting NPT 1/4" (part# B-4-ME; Swagelok®) connected to a brass female quick-connect body NPT 1/4" (part# B-QC4-B-4PF; Swagelok®) using thread tape. A precision drill is used to drill a single hole where the PVC pipe and end-cap overlap. The hole is threaded with a tap drill for attachment of the canister valve using sufficient thread tape. Before attachment of the valve, drilling debris is shaken out of the canister via the drill hole to avoid future valve blockage.

The number of canisters to manufacture relies on the number of experimental cows (including background cows) per study. The number of canisters required is at least three times the number of experimental cows per study.

Testing for leaks

Pressurise canister with dry air using a mobile air compressor attached with a male Swagelok quick release connector. Pressurised canister is slowly submerged in a container containing clear water, while checking for obvious and subtle air bubbles released from the submerged canister and noting the location of the leak. Connect leaky canister to the vacuum pump and apply contact adhesive to the location of the apparent leak while the canister is being evacuated to 98 kPa vacuum continuing for at least 2 min. After evacuating the canister, allow the amended canister to cure overnight and retest in the morning. When the leak comes from the quick-connection tighten the elbow/quick-connect body connection and retest. If the leak persists repeat the process, but when the leak repair fails for the third time discard the canister.

Flushing

Canisters should be flushed prior each use to overcome cross contamination imposed by residual gas in the canisters. This is done by implementing five cycles of evacuating to 98 kPa vacuum, filling with ultra-high purity nitrogen gas (999.99 g/kg nitrogen) and evacuating again to 98 kPa vacuum.

A.5 SAMPLING LINE AND RESTRICTOR

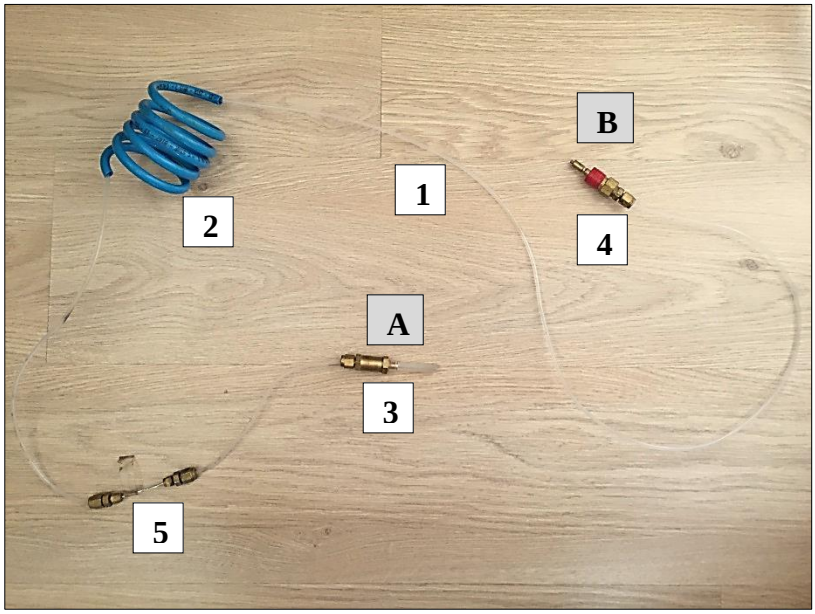

Figure A-4 Sampling line with in-line restrictor. Flow is from A to B.

Design

(1) flexible nylon tube 3 m long (1/8" OD, 1 mm wall thickness; Kriess Hydraulics CC.)

(2) blue polyurethane coiled tubing 1.5 m long when straightened (8 mm OD, 5 mm ID; Kriess Hydraulics CC). ***Pull coiled tube straight to insert nylon tube.***

(3) brass in-line particulate filter with complete nut and ferrule set to fit 1/8" OD tubing (15 µm; part# B-2F-15; Swagelok®, Ohio, USA). Silicone tube (50-70 mm long, 10 mm OD) is attached the inflow end of the filter to capture and avoid dewdrops from entering the filter during grazing. ***When installing the filter take note of the direction of the arrow on the filter. The arrow indicates the direction of flow and should point from A to B in Figure A-4***.

(4) brass quick-connect stem with complete ferrule and nut set to fit 1/8" OD tubing (part# B-QC4-D-200; Swagelok®) for attachment of canister.

(5) in-line flow restrictor consisting of 50 mm stainless steel tubing (1/16" OD, 0.2" ID; part# YY-RES-21503; LECO Africa (Pty) Ltd., Kempton Park, South Africa) attached with two reducing unions from 1/8" to 1/16" with complete ferrule and nut set (part# B-200-6-1; Swagelok®). ***Use tube cutting pliers to cut the stainless steel tubing (YY-RES-20193; LECO Africa (Pty) Ltd).***

Follow Swagelok guidelines for ferrule placement and correct tightening of nuts.

Make at least 20% extra complete sampling lines with flow restrictors above the selected number of experimental animals and ensure that this surplus is daily available throughout the SF_6 trial. This will serve as quick replacements for broken sampling lines or blocked flow restrictors.

Flow calibration

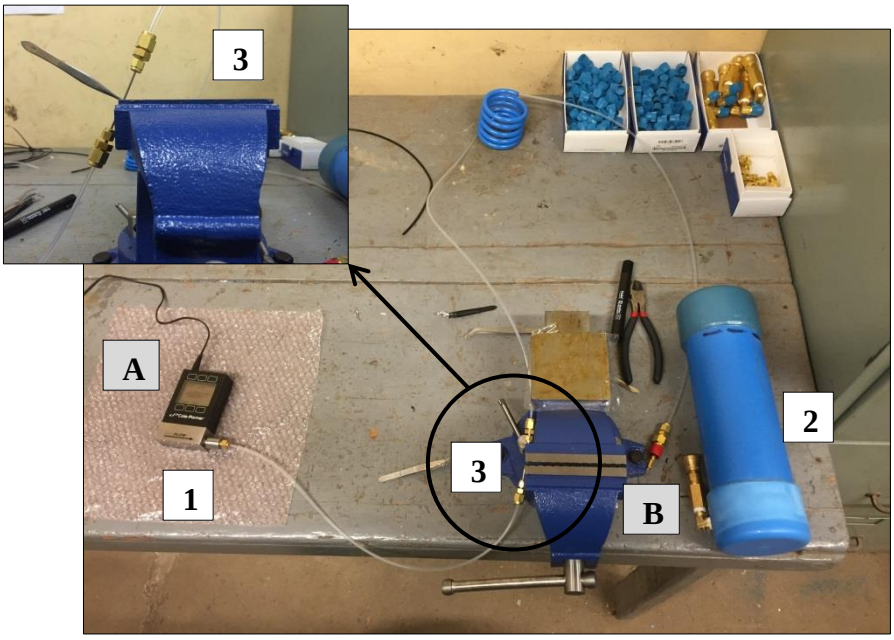

Figure A-5 Crimping of stainless steel tube to calibrate flow. Photo insert presents a close-up of the crimping procedure using tweezers and a table top vice-grip. Flow is from A to B.

(1) sampling line, with particulate filter removed, is connected to a gas flow meter (part# 32908-53; Cole Parmer, Vernon Hills, Illinois, USA) with a similar particulate filter attached on the inlet (not visible in Figure A-5). ***Note the direction of flow on the gas flow meter for correct connection.***

(2) male quick-connect of the sampling line is connected to an evacuated (98 kPa vacuum) canister.

(3) stainless steel tube is crimped using a table top vice-grip (with or without a tweezer) until desired flow is achieved as read from the gas flow meter. Evacuated canister should fill to 45-50% over a 24 h period to minimise sampling rate decline as canister vacuum declines. A flow rate of 0.53 to 0.59 mL/min will be required to fill a 1700 mL canister to 45-50% over a 24 h period. ***If the flow rate falls below 0.53 mL/min during the crimping procedure the table top vice-grip can be used to reverse the process by applying pressure on the sides of the crimped area.***

Halter

Figure A-6 (Left) side view of custom equine halter with nose leather platform for attachment of complete sampling line. **(Right)** close-up of the nose leather platform for attachment of the sampling line inlet close to the nostrils (particulate filter with silicone tube).

The halter in use is a commercially available nylon equine halter (large size) fitted with a custom leather nose platform with Velcro strap to secure the particulate filter (Figure A-6). The nose platform positions and secures the sampling line inlet close to the nostrils of the animal. ***Note that the open-end of the silicone tube (inlet) should be level with the nostril line of the cow when the cow's head is down in the grazing position and not when the cow's head is upright – the silicone tube should be cut to the correct length (each cow is different). This avoids that the inlet gets submerged while the animal drinks water or make contact with the concentrate while the animal is feeding, hence evading blockages.***

A single buckle is used to tighten the halter (located on the left hand side of the cow in Figure A-6). The halter should be tightened to prevent excessive movement of the nose platform but allowing restricted hand movement under the neck and chin strap. The sampling line is attached to the halter with four cable ties: one over the particulate filter, one on each side of the in-line flow restrictor, and one on the back of the neck of the animal. Furthermore, the in-line flow restrictor can be further secured with adhesive tape (not shown in Figure A-6).

A.6 BACK-MOUNTED HARNESS

See Chapter 6.

A.7 CLEANING AND STORAGE

After each trial all equipment should be cleaned and stored in a well ventilated storage room. Canisters should be flushed following the flushing procedure, therefore canisters are stored evacuated. Harness bodies are cleaned with a high pressure hose and hung to dry completely before long-term suspended storage. Halters are brushed with a hard brush to remove manure and animal hair debris before long-term suspended storage. Furthermore, the cleaning procedure of Williams et al. (2016) can be used to clean particulate filters and quick-release fittings.

A.8 RESOURCES

Table A-2 Detailed source description and estimated cost in South African Rand of the major items required for the design and functionality of the customised sulphur hexafluoride tracer gas (SF_6) technique for grazing dairy cattle implemented at the Outeniqua Research Farm, George, South Africa.

Item	Source	Source company	Item code	Est. cost per item or as stated
Permeation tube				
SF_6 Cylinder 9 kg	Local	AFROX	541601-IE-C	R 8 000
Gas hose	Local	AFROX	W002600, 5 mm DA, 3 m long	R 200
Brass mounting plate for cow brass body	Int'l (Ireland)	Precision Engineering, darragh.w@ptprecisioneng.com	-	R 10 000
Brass body	Int'l	Precision Engineering	9/16" diameter for Swagelok fitting	R 110
Nylon washer	Int'l	Precision Engineering	11 mm OD, 5.3 mm ID	R 8
Teflon discs	Int'l	Precision Engineering	0.2 mm thick and 1/2" OD	R 35
Stainless steel frit	Int'l	Precision Engineering	316 stainless steel 1/2" OD	R 75
Gelatine Capsules	Int'l (USA)	Torpac Inc., Cynthia@torpac.com	A-10 CT, Size 10 clear empty gelatin capsule (100 per box)	R 600
Incubator	Local	Air & Vacuum Technologies (Pty) Ltd., kzn&cpt@vactech.co.za	Temperature and Accuracy: 39°C±0.2°C	R 20 000
Canister				
Male Elbow, 1/4" Male NPT	Local	Swagelok	B-4-ME	R 250
Vacuum gauges	Local	SA Gauge, sales@sagauge.com	R3A63G14B -100+0 KPA / PSI	R 400
Quick Connect Stem 1/4", Female NPT	Local	Swagelok	B-QC4-D-4PF	R 430
Quick-Connect Body 1/4", Female NPT	Local	Swagelok	B-QC4-B-4PF	R 490
Vacuum pump	Local	Air & Vacuum Technologies (Pty) Ltd.	VWOR-RECIPE1, Rietschle Pump -	R 22 000

			VCB20 (1021302AA), 0.75 kW, 380V	
Quick-Connect Stem, 1/4"	Local	Swagelok	B-QC4-D-400, for vacuum pump	R 380
Quick Connect Stem, 1/8"	Local	Swagelok	B-QC4-D-200, for nitrogen cylinder	R 470
Sampling line				
Cole Parmer flowmeter	Local	Cole Parmer, salesjhb@labcon.co.za	32908-53; 0.05 to 5 ml/min	R 30 000
Coiled tubing	Local	Kriess Hydraulics CC (George)	Inner diameter should fit 1/8" tubing (1.5 m per cow)	R 50
Sampling line tubing	Local	Kriess Hydraulics CC	Flexible 1/8" OD, thick wall (3 m per cow)	R 40
Reducing Union, 1/8" x 1/16"	Local	Swagelok	B-200-6-1	R 125
Quick Connect Stem 1/8"	Local	Swagelok	B-QC4-D-200	R 470
Brass In-Line Particulate Filter, 1/8"	Local	Swagelok	B-2F-15, 15 micron	R 680
Capillary tube	Local	LECO Africa (Pty) Ltd., esrie@lecoafrica.co.za	YY-RES-21503, STAINLESS STEEL 1/16" OD x 0.2" ID (7.6 m batch)	R 1 900
Capillary tube cutter	Local	LECO Africa (Pty) Ltd.	YY-RES-20193, TOOL 1/16" TUBING CUTTER PLIERS	R 1 200
Gas sampling				
Dry air Cylinder 8.5 kg	Local	AFROX	13-RC	R 650
Piston Sub-sampler	Int'l (NZ)	NIWA, Ross.Martin@niwa.co.nz	-	R 90 000
Animal equipment				
Halter	Local	Any equine retailer	Nylon, large or extra-large size	R 250
Harness	Local	Any equine retailer	Nylon lunge roller with neoprene padding, large or extra-large size	R 400
Bespoke support shaft	Local	Franette Botha, medical orthotist prosthetist (practice no 0544744)	See Chapter 6 for detailed description	R 200

A.9 REFERENCES

Lassey, K.R., Walker, C.F., McMillan, A.M.S., Ulyatt, M.J., 2001. On the performance of SF_6 permeation tubes used in determining methane emission from grazing livestock. Chemosphere – Global Change Sci. 3, 367–376.

Williams, S.R.O., Moate, P.J. & Deighton. M.H., 2016. The modified SF_6 technique for the determination of methane emissions from dairy cows. Notes for a workshop held at Ellinbank, Victoria, Australia 10-11 February 2016. Department of Economic Development, Jobs, Transport and Resources, Melbourne, Victoria, Australia. pp. 1–56.