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

Josef D.V. van Wyngaard,* Robin Meeske,† and Lourens J. Erasmus*

*Department of Animal and Wildlife Sciences, University of Pretoria, Hatfield 0028, South Africa

†Western Cape Department of Agriculture, Outeniqua Research Farm, George 6530, South Africa

[1]Corresponding author: Josef de Villiers van Wyngaard; P.O. Box 249, George 6530, South Africa; +27 82 336 0626; jdvvanwyngaard@gmail.com

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 (9 rumen-cannulated) were used in a randomized 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 3 cannulated cows per treatment). Enteric CH_4 emissions were measured from 11 cows per treatment group during one 7-d measurement period using the sulfur 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 fiber disappearance decreased linearly. Acetic and propionic acid were unaffected by treatment, while butyric acid (5.21 to 6.14 m*M*/L) 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: pasture-based, methane mitigation, SF_6, tropical pasture

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 favor 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 utilized 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 hypothesized 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.

MATERIALS AND METHODS

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 characterized 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.

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 were 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 1 of 3 treatment groups on January 25, 2017. Additionally, 9 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 3 groups. Each treatment group (consisting of 20 cows) was then randomly assigned to 1 of 3 treatments. Treatments consisted of 3 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 × 106 IU of vitamin A/kg, 1 × 106 IU of vitamin D3/kg, and 8 × 103 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).

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 herbage masses were estimated using the following site-and-season-specific linear regression equation: Herbage mass (kg of DM/ha) = [87.8 × sward height (rising plate

meter reading)] – 32.7 (R^2 = 0.94). According to this equation, a residual herbage mass of 933 kg of DM/ha was attained at the target post-grazing height of 5.5 cm aboveground.

Measurements

Animal performance. All cows were milked twice daily at 0700 h and 1500 h in a dairy parlor 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 analyzer (FOSS Analytical, DK-3400 Hillerød, Denmark) for determining milk fat, milk protein, milk lactose and MUN, and a Fossomatic FC (FOSS Analytical) for determining SCC. Fat corrected milk standardized 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 2 consecutive days and BCS recorded before afternoon milking at the start and the end of the study period. Bodyweight 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).

DMI. Individual pasture DMI of intact cows was estimated with the use of titanium dioxide (TiO_2) as an external marker to determine fecal 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 1 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. fecal samples on d 5 to d 10) of Pinares-Patiño et al. (2008) was implemented from March 22 to 31, 2017. Fecal samples were immediately oven dried (65°C, 72 h), pooled within-animal and analyzed for TiO_2 concentration by the method of Myers et al. (2004). Fecal output was calculated from the daily TiO_2 dose and TiO_2 concentration in feces 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 5 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 fecal samples were determined according to Krizsan et al. (2015) by incubating samples in sacco 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 Ankom2000 fiber analyzer (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 $_{feces}$ (kg/kg)] − iNDF $_{concentrate}$ intake (kg/d)]/iNDF $_{forage}$ (kg/kg).

Enteric CH_4. 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 7 consecutive days (March 26 to April 2) to ensure at least 4 representative 24 h gas samples per cow after the completion of the measurement period. Empty permeation tubes (P&T Precision Engineering Ltd., Naas, Co. Kildare, 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, Ferndale, Johannesburg, South Africa) set at 39.0°C for 4 wk weighing the tubes (Sartorius BP210S, Sartorius AG, Goettingen, 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 gelatin capsule (Torpac Inc., Fairfield, NJ, 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, USA) cut to 50 mm length and crimped using a table top vice-grip until the specified flow was attained.

Background (ambient) emissions 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 (analyzed approximately 14 d after sampling).

Gas samples were analyzed using an automated gas analyzer equipped with a Gilson Sample Changer (Gilson, Inc., Middleton, WI 53562-0027, USA) modified at NIWA to analyze pressurized 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).

Rumen Fermentation. Ruminal pH, fermentation end-products, and in sacco pasture DM and NDF disappearance were determined using the 9 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 4 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 analyzed for DM content (AOAC, 2000; method 934.01), NDF content (as described before), and ADL content (Goering and van Soest, 1970).

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 5 cm aboveground from the successive grazing-strip. Samples were thoroughly homogenized, 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 analyzed for DM, ash, CP (N content determined

using a LECO TrumacTM 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. The ADF (using the Ankom2000 fiber analyzer) and ADL content were determined according to Goering and van Soest (1970). Samples were also analyzed for GE (MC–1000 modular calorimeter, Energy Instrumentation, Sandton, South Africa; operator's manual) and in vitro OM digestibility (IVOMD; Tilley and Terry, 1963; using rumen fluid from a rumen-cannulated SA Mutton Merino ram fed good-quality alfalfa hay). The ME was calculated using the equations of MAFF (1984): $ME_{concentrate} = 0.84 \times GE \times IVOMD$ and $ME_{pasture} = 0.81 \times GE \times IVOMD$. Mineral composition was determined according to the procedure of AgriLASA (1998; method 6.1.1).

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 analyzed as a randomized 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 2 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 sacco disappearance and rumen fermentation end-products were analyzed as a triplicated 3 × 3 Latin square with ANOVA 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 analyzed using the statistical program GenStat (Payne et al., 2014).

RESULTS

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 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.

Table 1. Chemical composition of the concentrate offered and of the pasture offered averaged over both the 7-wk study period and CH_4 measurement period

Item	Concentrate (n = 6)	Pasture	
		7-wk study (n = 6)	CH_4 measurement period (n = 5)
Initial DM (%)	89.9	17.8	17.3
DM composition (g/kg of DM)			
CP	133	208	193
EE	36.7	30.1	26.0
NDF	107	574	591
ADF	39.4	293	299
Ash	78.3	104	114
OM digestibility (g/kg of DM)	946	740	627
GE (MJ/kg of DM)	17.2	18.0	17.7
ME (MJ/kg of DM)[1]	13.7	10.8	8.98
Mineral composition			
Ca (g/kg of DM)	15.6	4.61	3.85
P (g/kg of DM)	5.95	4.20	3.42
Mg (g/kg of DM)	4.40	4.97	5.07
K (g/kg of DM)	9.70	26.1	42.9
Na (g/kg of DM)	2.27	8.25	1.86
Mn (mg/kg of DM)	82.6	34.7	49.8
Cu (mg/kg of DM)	27.7	7.85	7.58
Fe (mg/kg of DM)	110	95.7	98.7
Zn (mg/kg of DM)	130	54.9	57.2

[1]Calculated.

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). Cows were offered pasture at 11.7 kg of DM/cow per day above 5 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.

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

Item	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)[1]		
Pre-grazing	2197 ± 653.1	2027 ± 256.4
Post-grazing	1008 ± 172.0	1082 ± 115.1
DHA[2] (kg of DM/cow per day)	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/cow per day)	6.11 ± 1.646	5.22 ± 0.917

[1]Estimated 5 cm aboveground using a rising plate meter.

[2]Daily herbage allowance.

Milk production, Milk composition and Cow Condition

Milk production and cow condition measurements over the 7-wk study period are presented in Table 3. Milk yield, including FCM and ECM, were affected by treatment and increased linearly and quadratically ($P < 0.05$) with increasing concentrate feeding level. Similarly, milk fat and milk lactose yield also increased linearly and quadratically ($P < 0.05$) while milk protein yield only increased linearly ($P < 0.001$) with increasing concentrate feeding level. 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. 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

Table 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 (n = 17)

	Concentrate level (kg/d as fed)				*P*-value[2]		
Item	0	4	8	SEM	Con	Lin	Quad
Milk yield (kg/cow per day)	9.03^{c}	14.0^{b}	17.7^{a}	0.239	<0.001	<0.001	0.035
FCM yield (kg/cow per day)	11.4^{c}	17.4^{b}	20.8^{a}	0.32	<0.001	<0.001	0.003
ECM yield (kg/cow per day)	11.2^{c}	17.1^{b}	20.7^{a}	0.29	<0.001	<0.001	0.002
Milk fat (g/kg)	58.3^{a}	56.7^{ab}	52.2^{b}	1.46	0.016	0.006	0.43
Milk protein (g/kg)	38.0	37.3	37.6	0.51	0.60	0.56	0.42
Milk protein to fat ratio	0.66^{b}	0.66^{b}	0.73^{a}	0.016	0.004	0.002	0.13
Milk lactose (g/kg)	44.6^{b}	46.3^{a}	46.5^{a}	0.27	<0.001	<0.001	0.036
Milk solids[1] (g/kg)	141	140	136	1.7	0.12	0.056	0.42
MUN (mg/dL)	15.5^{a}	16.2^{a}	13.6^{b}	0.36	<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/cow per day)	0.52^{c}	0.79^{b}	0.91^{a}	0.019	<0.001	<0.001	0.005
Milk protein yield (kg/cow per day)	0.34^{c}	0.52^{b}	0.66^{a}	0.010	<0.001	<0.001	0.17
Milk lactose yield (kg/cow per day)	0.40^{c}	0.65^{b}	0.82^{a}	0.014	<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

[1]Milk solids = milk fat + milk protein + milk lactose.

[2]Con: contrast; Lin: linear; Quad: quadratic.

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.

DMI and enteric CH_4 emissions

The effect of concentrate feeding level on DMI and CH_4 emissions, along with the milk production and milk composition recorded during this measurement period, are presented in

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

	Concentrate level (kg/d as fed)				*P*-value[4]		
Item	0	4	8	SEM	Con	Lin	Quad
BW (kg)	391	389	396	7.5	0.79	0.64	0.63
Fecal output (kg/cow per day)	3.36	2.82	2.78	0.228	0.16	0.087	0.38
Intake							
Pasture DMI (kg/cow per day)	11.2[a]	9.19[ab]	8.36[b]	0.704	0.028	0.010	0.50
Total DMI (kg/cow per day)	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
GEI[1] (MJ/cow per day)	202[b]	228[b]	275[a]	12.7	0.002	<0.001	0.50
MEI[2] (MJ/cow per day)	121[c]	149[b]	189[a]	7.6	<0.001	<0.001	0.50
Feed efficiency (kg of ECM/kg of 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/cow per day)	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
Ym[3] (%)	8.91	8.97	7.85	0.341	0.052	0.039	0.17

[1]Gross energy intake.

[2]Metabolizable energy intake.

[3]CH_4 energy per gross energy intake.

[4]Con: contrast; Lin: linear; Quad: quadratic.

Table 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.

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.

Rumen Fermentation

The effect of concentrate level on diurnal patterns of ruminal pH is presented in Figure 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 ruminal 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. Thereafter the 4 kg group recovered to a similar ruminal 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 ruminal pH compared with the 0 kg group.

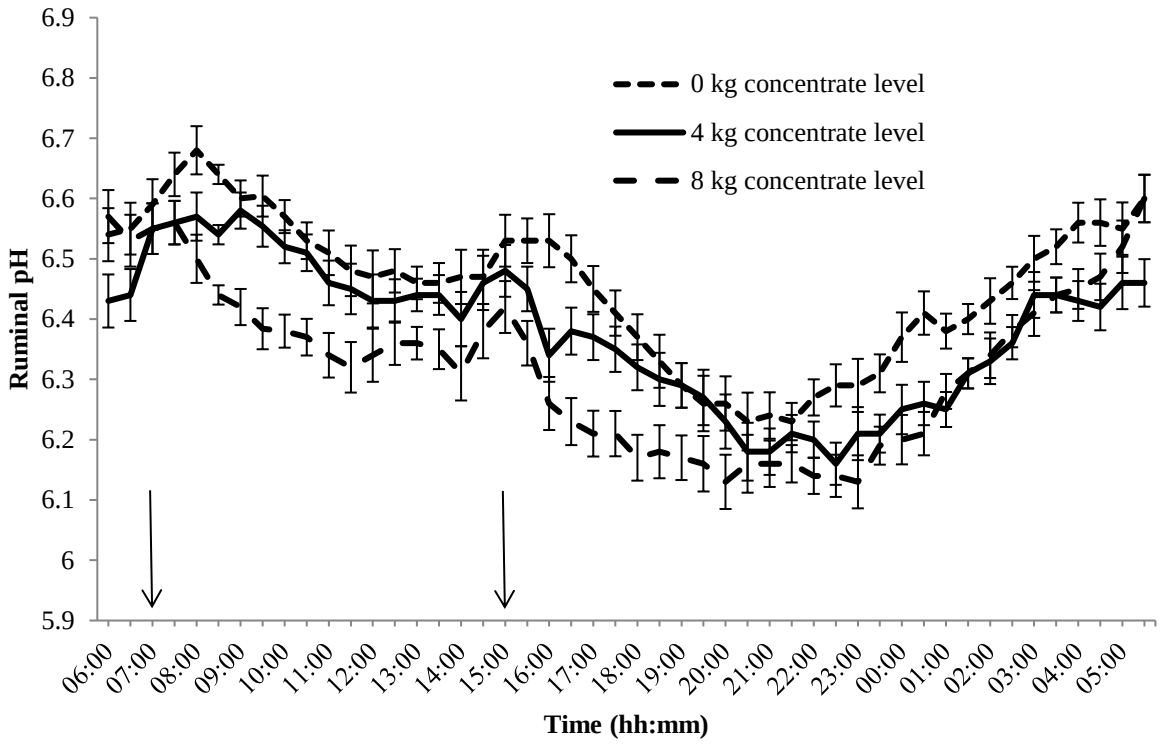

Figure 1. The effect of concentrate supplementation level on diurnal ruminal pH of early lactation Jersey cows grazing kikuyu-dominant pasture in late summer (n = 9). Error bars indicate SEM and arrows indicated when concentrate was fed

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 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. 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

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

	Concentrate level (kg/d as fed)				*P*-value[2]		
Item	0	4	8	SEM	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	1.015	0.43	0.32	0.40
pH 6.0	1.67	1.33	3.39	1.175	0.44	0.32	0.43
pH 6.2	5.17	6.61	5.83	1.197	0.70	0.70	0.47
pH 6.4	10.9	12.6	13.2	1.71	0.63	0.37	0.81
pH 6.6	15.1	19.2	20.3	1.74	0.13	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*)	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 (coefficient)							
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 (coefficient)							
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[1]							
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

[1]Rate of NDF disappearance.

[2]Con: contrast; Lin: linear; Quad: quadratic.

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.

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 in dairy and beef cattle (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

utilized 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-utilized (Fulkerson et al., 1999).

Between the two extreme treatments, pasture DMI decreased with increasing concentrate feeding level while total DMI, GE intake and ME 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 utilized 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 feeding level, respectively, while a marginal milk response (4 vs 8 kg concentrate level) of 1.03 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 feeding 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 utilizing 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 seems 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 reflects 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 where supplemented with a fiber-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 fiber 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 favors 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 favor 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 favor propionate production, but rather favorable for butyrate production.

CONCLUSIONS

Enteric CH_4 emissions were measured from lactating Jersey cows grazing medium 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 studies to better understand rumen fermentation proxies for CH_4 emissions, and for meta-analysis studies in developing robust prediction equations; hence, improving GHG inventories.

ACKNOWLEDGEMENTS

This research was supported and funded by the Western Cape Department of Agriculture (Elsenburg, South Africa) and the Western Cape Agricultural Research Trust (Elsenburg, South Africa). A special thanks goes out to Heike Lorenz (Christian-Albrechts-Universität Kiel, Institute of Crop and Plant Breeding, Grassland and forage production/organic farming, Kiel, Germany) for support throughout the pasture DMI and CH_4 measurement period. The financial assistance of the National Research Foundation (NRF; Pretoria, South Africa) towards this research is hereby also acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

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