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

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Highlights

- Dietary nitrate as methane mitigation strategy for grazing dairy cows
- Concentrate DMI and milk yield decreased with nitrate addition
- Total DMI was unaffected by treatment
- Methane production and yield tended to decrease with nitrate addition
- Ruminal pH fluid and total VFA concentration increased with nitrate addition

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.

Keywords: electron receptor; kikuyu; methane mitigation; SF_6

Abbreviations: BCS, body condition score; CH_4, methane; CP, crude protein; DM, dry matter; DMI, dry matter intake; ECM, energy corrected milk; FCM, fat corrected milk; FO, faecal output; GE, gross energy; iNDF, indigestible neutral detergent fibre; ME, metabolisable energy; MUN, milk urea nitrogen; N, nitrogen; NDF, neutral detergent fibre; NIWA, National Institute of Water and Atmosphere; SCC, somatic cell count; SF_6, sulphur hexafluoride; TMR, total mixed ration; VFA, volatile fatty acid

1. Introduction

Methanogenesis is a natural process in the rumen where enteric methane (CH_4) and water are 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 feasible 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 protection/encapsulation of nitrate to slow the release of nitrate (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 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.

2. Materials and methods

2.1 Location description

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.

2.2 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 1). Limestone ($CaCO_3$) and urea were decremented as the inclusion of the nitrate source increased.

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

Parameter	Concentrate treatment		
	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
Salt	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.

2.3 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 ($R^2 = 0.84$).

2.4 Measurements

2.4.1 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).

2.4.2 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).

2.4.3 Enteric methane

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 for 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).

2.4.4 *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).

2.5 *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).

2.6 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).

3. Results

3.1 Feed composition and pasture measurements

The chemical composition of the dairy concentrate and pasture offered averaged across the 8-wk study period are presented in Table 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).

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 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 2. Chemical composition (g/kg of dry matter, 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

Parameter	Concentrate treatment (n = 4)			Pasture[3] (n = 18)
	Control	Low Nitrate	High Nitrate	
Initial DM[1] (g/kg)	909 ± 4.9	902 ± 3.1	904 ± 0.2	174 ± 21.5
DM composition (g/kg of DM or as stated)				
Crude protein	144 ± 0.1	146 ± 0.1	140 ± 0.3	192 ± 19.9
Nitrate	0[4]	11	23	3.2 ± 1.07
Ether extract	29 ± 2.8	21 ± 0.9	21 ± 0.1	25 ± 3.1
Neutral detergent fibre	146 ± 10.1	119 ± 10.3	120 ± 5.8	584 ± 35.9
Acid detergent fibre	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
In vitro organic matter digestibility	974 ± 14.9	993 ± 10.9	991 ± 4.5	650 ± 89.3
Gross energy (MJ/kg of DM)	17.1 ± 0.07	17.1 ± 0.02	16.6 ± 0.08	17.8 ± 0.28
Metabolisable energy[2] (MJ/kg of DM)	14.0 ± 0.25	14.2 ± 0.17	13.8 ± 0.13	9.38 ± 1.37
Mineral composition (g/kg of DM or as stated)				
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.
[2] Calculated (MAFF, 1984).
[3] Pasture–kikuyu (*Pennisetum clandestinum*) dominant.
[4] Sample represents four pooled concentrate samples.

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

Parameter	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)[1]		
Pre-grazing	2252 ± 408.3	2095 ± 419.4
Post-grazing	868 ± 113.2	871 ± 93.0
Daily herbage allowance (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; Estimated 3 cm aboveground level using a rising plate meter; Pasture yield (kg of DM/ha) = (90 × rising plate meter height reading) – 232 ($R^2 = 0.84$).

3.2 *Milk yield, 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). 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

Table 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	SEM[5]	P-value		
Parameter[1]	Concentrate treatment[4]						
	Control	Low Nitrate	High Nitrate		Contrast	Linear	Quadratic
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[2] (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[3]	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] Means in the same row with different superscripts differ ($P<0.05$).

[1] FCM–4% fat corrected milk (calculated); ECM–energy corrected milk (calculated); MUN–milk urea nitrogen; SCC–somatic cell count; BW–body weight; BCS–body condition score.

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

[3] Scale 1 to 5.

[4] Concentrate feeding level: 5.4 kg of dry matter (DM)/cow per day split in two equal portions during milking (0530 h and 1330 h); nitrate inclusion levels: 0, 11 and 23 g/kg of DM for the control, low nitrate and high nitrate concentrates, respectively.

[5] SEM–standard error of mean.

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.

3.3 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 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.

Table 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				
Parameter[1]	Concentrate treatment[2]			SEM[3]	P-value		
	Control	Low Nitrate	High Nitrate		Contrast	Linear	Quadratic
Faecal output (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] Means in the same row with different superscripts differ ($P<0.05$).

[1] 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 (calculated); Y*m*–CH_4 energy per GEI.

[2] Concentrate feeding level: 5.4 kg of dry matter (DM)/cow per day split in two equal portions during milking (0530 h and 1330 h); nitrate inclusion levels: 0, 11 and 23 g/kg of DM for the control, low nitrate and high nitrate concentrates, respectively.

[3] SEM–standard error of mean.

3.4 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 (Fig.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 were 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 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.

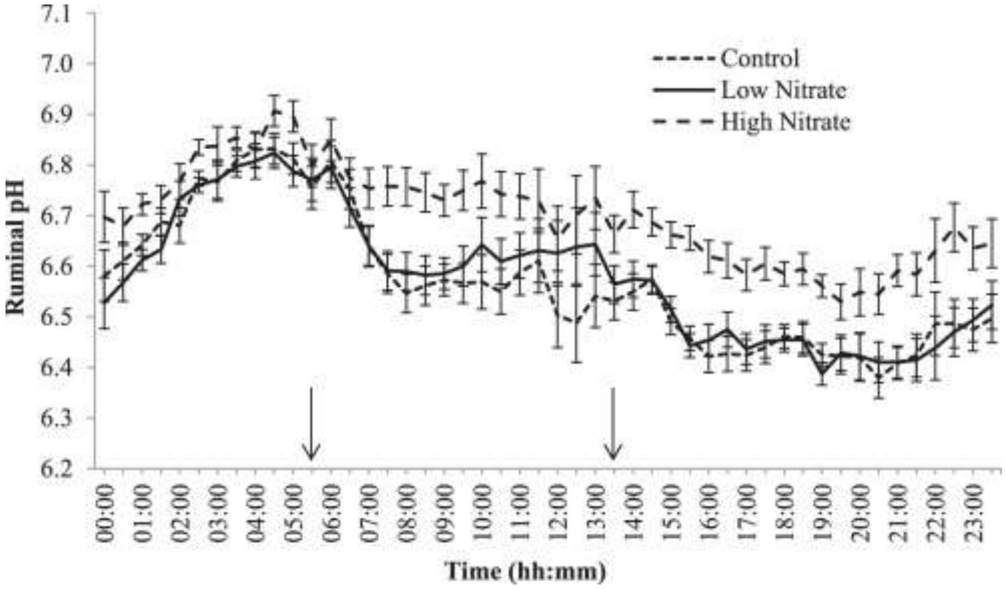

Fig. 1. Diurnal ruminal pH of early lactation Jersey cows (rumen-cannulated) grazing kikuyu-dominant pasture in late-summer fed concentrates containing zero, low and high levels of nitrate (n = 6). Concentrate feeding level: 5.4 kg of dry matter (DM)/cow per day split in two equal portions during milking (0530 h and 1330 h); nitrate inclusion levels: 0, 11 and 23 g/kg of DM for the control, low nitrate and high nitrate concentrates, respectively. Error bars indicate SEM and arrows indicate when concentrate was fed

Table 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 (mean of the rumen measurement periods)

Number of cows	6	6	6	SEM[3]	P-value		
Parameter[1]	Concentrate treatment[2]						
	Control	Low Nitrate	High Nitrate		Contrast	Linear	Quadratic
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	0.394	0.42	0.23	0.68
pH 6.2	1.83	1.92	0.50	0.795	0.42	0.28	0.47
pH 6.4	4.83	5.25	2.33	0.709	0.054	0.047	0.10
pH 6.6	12.4	12.6	5.92	1.770	0.061	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 (coefficient)							
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 (coefficient)							
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} Means in the same row with different superscripts differ ($P<0.05$).

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

[2] Concentrate feeding level: 5.4 kg of dry matter (DM)/cow per day split in two equal portions during milking (0530 h and 1330 h); nitrate inclusion levels: 0, 11 and 23 g/kg of DM for the control, low nitrate and high nitrate concentrates, respectively.

[3] SEM–standard error of mean.

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.

4. 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 would 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 line 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 suggests that hydrogen may have 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 are 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 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 properties 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.

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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