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### 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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#### 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<sub>4</sub> 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<sub>4</sub> emissions and cow production performance. Additionally, six rumen-cannulated cows in a replicated  $3 \times 3$ 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<sub>4</sub> emissions and total dry matter intake (DMI) from 11 cows per treatment were measured during one 6d measurement period using the sulphur hexafluoride tracer gas technique. Individual pasture DMI was determined using TiO<sub>2</sub> 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 (5.5–3.7 kg/d) while pasture DMI increased linearly (9.1–11.4 kg/d) with increasing dietary nitrate addition. Methane production (313-280 g/d), CH<sub>4</sub> yield (21.8-18.7 g/kg of DMI) and  $CH_4$  energy per gross energy intake (6.9–5.9%) 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 (6.2-6.3) tended to increase while total volatile fatty acid (VFA) concentration (99.9-104 mM/L) increased quadratically with increasing dietary nitrate addition. Individual VFA concentrations were unaffected by treatment. Rate of NDF disappearance (2.4-2.8%/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<sub>4</sub> emissions while improving the

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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<sub>6</sub>, sulphur hexafluoride; TMR, total mixed ration; VFA, volatile fatty acid

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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.

#### 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 1961–2016 (FAO, 2016), the need to abate  $CH_4$ emissions from ruminants is increasing.

Nitrate, an electron receptor, has been labelled as a promising CH<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> production from dairy cows, of which all were total mixed ration (TMR)-based and utilised respiration chambers to measure CH<sub>4</sub> emissions (van Zijderveld et al., 2011; Lund et al., 2014; Petersen 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 asphysia 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<sub>4</sub> emissions, production performance and rumen fermentation of Jersey cows grazing kikuyu-dominant pasture during late-summer. We hypothesised that CH<sub>4</sub> 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.

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