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Effects of prolonged oral administration of dicyandiamide to dairy heifers on excretion in urine and efficacy in soil

B.G. Welten[a,*], S.F. Ledgard[a], L.A. Schipper[b], J.E. Waller[a], M.J. Kear[a], M.M. Dexter[a]

[a] AgResearch Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand
[b] Department of Earth and Ocean Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand

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ABSTRACT

Oral administration of the nitrification inhibitor dicyandiamide (DCD) to grazing ruminants for excretion in urine represents a targeted mitigation strategy to reduce nitrogen (N) losses from grazed pastures. A field trial and allied laboratory incubation study were conducted to examine the effects of oral administration of DCD to non-lactating Friesian dairy heifers on excretion of DCD in urine and efficacy in soil. Dairy heifers were orally administered DCD daily at three treatment levels (low, medium and high; 12, 24 and 36 g DCD heifer^{-1} day^{-1}, respectively) and compared to a nil-DCD control group over a 90-day continuous dosing period. There were no adverse effects of DCD administration on heifer health or growth, as inferred by live-weight gain and measured blood metabolite levels. Prolonged administration of DCD to dairy heifers resulted in the sustained excretion of DCD in the urine over 90 days and inhibition of nitrification of urinary-N in the silty peat soil for up to 56 days (incubated at 20 °C; $P < 0.001$). Field soil sampling (0–75 mm depth) of individual urine patches for DCD analysis revealed that a 3-fold increase in the rate of DCD administered resulted in a similar increase in the concentration of DCD voided in the urine and subsequently deposited in urine patches (median equivalent DCD application rates of 22, 36 and 59 kg ha^{-1} for the low, medium and high DCD treatment levels, respectively; $P < 0.001$). However, large differences (up to 40-fold) existed between individual urine patches in the rate of DCD deposited at each treatment level, which showed a positively skewed distribution. This study highlights the viability of prolonged daily administration of DCD to ruminants for sustained excretion in urine and effective inhibition of nitrification in soil as a practical targeted mitigation technology to reduce urinary-N losses from grazed pastures.

1. Introduction

Loss of surplus nitrogen (N) from agricultural systems through nitrate leaching and gaseous emissions of ammonia and nitrous oxide (N_2O) has deleterious effects on the environment (Galloway et al., 2004; Robertson and Vitousek, 2009). Agricultural systems contribute approximately 35% of the annual N_2O emissions worldwide and intensively grazed pastures have been shown to lose between 30 and 200 kg N ha^{-1} year^{-1} through nitrate leaching (Kroeze et al., 1999; Ledgard, 2001). Urine deposition by ruminant animals in localised patches is equivalent to N loading rates of approximately 500–1000 kg N ha^{-1} and has been identified as the predominant source of N loss from grazed pastures (Haynes and Williams, 1993).

One management strategy that is effective in reducing urinary-N losses from grazed pastures is the surface application of the nitrification inhibitor, dicyandiamide (DCD), to the soil to retard the activity of the nitrifying bacteria, inhibiting the conversion of ammonium-N (NH_4^+-N) to nitrate-N (NO_3^--N) in soil (Amberger, 1989; Di et al., 2010). In New Zealand, DCD is currently commercially applied by broadcasting DCD (10 kg ha^{-1}) over the entire grazed area (covering both urine and inter-urine areas) twice per year in late-autumn and winter within 7 days of grazing (Di et al., 2007). Oral administration of DCD to ruminant animals and subsequent excretion in the urine (Ledgard et al., 2008), is an alternative technique to surface broadcast application of DCD as a strategy to mitigate urinary-N losses. A particular advantage of this delivery mechanism is that this technique specifically targets individual urine patches which can cover up to 6% of the area in a single grazing (or about 23% annually; Moir et al., 2011). By specifically targeting each urine patch a lower total amount of DCD is potentially required to contact urine patches compared to broadcast application of DCD over the entire grazed area.

Early research investigated the use of supplementing DCD to ruminant animals as a possible non-protein N source in feeds. Davis et al. (1956) and Rust et al. (1956) supplemented DCD to

* Corresponding author. Tel.: +64 7 838 5273; fax: +64 7 838 5117.
E-mail address: brendon.welten@agresearch.co.nz (B.G. Welten).

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lactating dairy cows as a feed additive for up to 196 days and found no significant effect on milk production and measured lowered milk and blood urea-N levels, thereby concluding that DCD was not effectively utilised by dairy cows as a non-protein N source in feeds. More recently, Ledgard et al. (2008) showed that sheep excreted 86% of the administered DCD in urine. Their indoor metabolism stall study (5 days DCD treatment and 9 days withholding) demonstrated that regular administration of DCD was required to maintain effective levels of DCD in urine to inhibit nitrification during the main period of N loss (e.g. autumn–winter period; approximately 90 days). From this short-term indoor study, it was uncertain whether there was any direct prolonged effect on DCD excretion in the urine and efficacy in inhibiting nitrification of urinary-N in the soil.

There are a number of potential methods of administering DCD to ruminants including daily oral drenching, delivery in feed supplements and controlled release delivery systems in the rumen (Ledgard et al., 2008). Studies with DCD supplementation in water troughs for dairy cows showed a reduction in nitrate leaching and nitrous oxide emissions by approximately 40% under grazing (Ledgard et al., 2012). However, concentrations of DCD in the urine varied by more than an order of magnitude between individual sheep (Ledgard et al., 2008). This suggests that at a paddock scale there would be a mosaic of urine patches deposited onto the soil varying in DCD concentration. Research is therefore required to define the rate and distribution pattern of DCD deposited in urine patches over a range of DCD treatment levels to aid in the development of optimum DCD delivery strategies for ruminant animals.

The objectives of this study were to examine the effect of daily administration of DCD to dairy heifers at three treatment levels over a 90-day period on the (i) temporal changes in DCD concentration in urine and blood plasma (as an indicator of DCD transformation to body fluids e.g. urine), (ii) deposition of DCD in urine patches, and (iii) efficacy of DCD voided in urine to inhibit nitrification in soil following prolonged administration.

2. Methods

2.1. Site description

The study was undertaken at the AgResearch Ruakura Research farm located in Hamilton, Waikato region, New Zealand (37° 46′ 02″ S; 175° 19′ 20″ E). Pasture was comprised predominantly of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), established on a Motumaoho shallow silty peat soil (NZ classification: Acid Humic Organic Soil; USDA: Terric Medisaprist). The soil is widely distributed throughout the Waikato region and is formed in layers of decomposed peat with silty volcanic ash on alluvium with clay. The soil is moderately well drained using artificial drainage. A meteorological station located on site was used to continuously monitor rainfall and air temperature.

2.2. Animal management and treatment administration

All animal manipulations were conducted with approval from the Ruakura Crown Research Institute Animal Ethics Committee. A total of 48 Friesian dairy heifers (ca. 12 months old; 229.0 (SE 2.4) kg initial live-weight) were blocked by live-weight and randomly assigned to four groups. All trial animals exhibited blood metabolite parameters consistent with normal renal and hepatic function prior to the commencement of the trial (data not presented).

A randomised experimental block design was used with the following four treatments randomly allocated to the four groups (12 dairy heifers per group):

(1) control – nil (no DCD solution);
(2) low DCD – 12 g DCD dissolved in 500 ml water daily;
(3) medium DCD – 24 g DCD dissolved in 1000 ml water daily; and
(4) high DCD – 36 g DCD dissolved in 1500 ml water daily.

These dose rates were equivalent to 0.05, 0.10 and 0.15 g DCD kg^{-1} initial live-weight day^{-1} for the low, medium and high DCD treatment levels, respectively. The assigned treatments were administered daily to individual dairy heifers between 0800 and 0900 h for the 90-day treatment period (9th September – 7th December 2009), followed by a 7-day withholding clearance period (days 91–97; no treatments administered). On each day of treatment, DCD treated animals were restrained in a head bale and were orally administered the aqueous solution of DCD (>99% purity; Sigma–Aldrich New Zealand Ltd., Auckland, New Zealand) using an electronic drench unit (Drenchmatic model RD2001; ISL Animal Health, Hamilton, New Zealand). One stock solution of DCD (24.0 g DCD L^{-1}) was prepared daily for the DCD treated groups and was administered in 250 ml aliquot doses to give the required volume.

Treatment groups were rotationally grazed as a single herd according to typical New Zealand farm management and were fed fresh pasture and had free access to fresh water throughout the duration of the study.

2.3. Herbage sampling and analysis

On five occasions during the treatment period (days 3, 31, 50, 71 and 87) herbage was collected for nutritional analysis from a representative area of the paddock prior to grazing. Herbage was sampled using hand clippers to a standing height of approximately 35 mm (1500 kg DM ha^{-1}). Herbage samples were dried at 65 °C for 72 h to determine dry matter (DM) content, and finely ground (<150 μm) prior to nutritional analysis for crude protein content and metabolisable energy, using near infra-red spectroscopy (NIRS Bruker Optics MPA NIR spectrophotometer, Ettlingen, Germany).

2.4. Animal live-weight measurements

Animal live-weights were recorded using digital weigh scales (Tru-Test model SR2000, Tru-Test Ltd., Auckland, New Zealand) at weekly intervals at 0800 h prior to daily treatment and provision of grazing fresh pasture throughout the administration period.

2.5. Blood sampling

Duplicate serum and plasma samples were collected from each dairy heifer by jugular venipuncture into evacuated sterile tubes (Serum and K-EDTA tubes, Vacuette® 9 ml Tubes, Greiner Bio-One GmbH, Austria). Blood samples were collected at 0700 h prior to treatment application on days 8, 15, 28, 42, 58, 70 and 90 during the treatment period, and on days 91, 92, 93, 94 and 97 after withholding DCD administration. The plasma was immediately separated and stored at −20 °C prior to DCD analysis. The remaining plasma and serum samples were analysed for changes in selected biochemistry and haematology parameters (aspartate aminotransferase, creatinine kinase, gamma-glutamyl transferase, glutamate dehydrogenase, haemoglobin, hematocrit, mean corpuscular haemoglobin concentration, total erythrocyte and leucocyte cell counts, platelet count, lymphocytes, segmented neutrophils, eosinophils and basophils; New Zealand Veterinary Pathology Ltd., Hamilton, New Zealand).