Effects of a daily dose of zeolite on rumen ammonia of lactating dairy cows consuming fresh-cut ryegrass pasture

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Abstract

Zeolites are aluminosilicates that can be safely fed to ruminants on a daily basis. They have a strong affinity for cations, including ammonium (NH_4^+), which creates the potential to adsorb excess ammonium released in the rumen and reduce urinary nitrogen (N) excreted. The main objective of this proof-of-concept study was to evaluate the effect of a single dose of 300 g of zeolite, deposited into the rumens of cannulated dairy cows before the main morning meal of fresh-cut ryegrass pasture, on changes in rumen ammonium concentrations over the following 8 h. The two-week trial was a cross-over design with 12 cows, either fed ryegrass as a sole diet (Control) or with zeolite (Zeolite). There was a consistent trend for rumen ammonium concentrations in the zeolite-treated cows to increase more slowly over time, and were lower than control cows 8 h after the zeolite was given (P = 0.08). This study provides some indications that a daily dose of zeolite, fed at the onset of the morning meal, may have a moderating effect on rumen ammonium concentration, and reduce plasma urea N concentrations in lactating dairy cows fed a ryegrass-dominant diet.

Keywords: cannulated cows; N metabolism; N mitigation; plasma urea N

Introduction

Nitrogen utilization for milk production by grazing dairy cows ranges from about 14-25% of dietary N intake. The lowest values are associated with pastures containing high concentrations of crude protein (CP), but are exacerbated by low milk production (Waghorn et al. 2007; Pacheco & Waghorn 2008). In pastoral dairying systems, approximately 80% of the urinary N is discharged onto pastures (McLeod et al. 2009), with the remainder on races, feeding pads and yards. Up to 55% of urinary N on pastures can leach, depending on the N load per urine patch (kg/ha) and the time of year (Romera et al. 2017). Under New Zealand conditions about 2% of urinary N can be lost as nitrous oxide, depending on conditions, such as soil moisture (de Klein & Ledgard 2005). These losses demonstrate the need to evaluate feeding-management strategies to reduce urinary N excretion.

Compounds, such as condensed tannins, added to the animal diet, have the potential to reduce N leaching losses by shifting the site of excreted N from urine into the faeces, thereby reducing the amount of N excreted in urine (Selbie et al. 2015). However, care should be taken to avoid reductions in intakes or digestibility.

Zeolites are aluminosilicates that have a porous structure with a very large surface area (120-130 m^2/g) (www.optimate.co.nz), that enables absorption, release and exchange of chemicals, nutrients and toxins. Clinoptilolite and Mordenite are two types of zeolites occurring in natural abundance especially in volcanic areas, including parts of New Zealand. These have a high ion exchange capacity (100-120 meq/100g) and affinity for ammonium ions and other cations (K^+, Mg^+, Ca^+, Na^+; Weatherly & Miladinovic 2004). New Zealand zeolites are unique in that they are geologically young, free from amorphous material, have a particularly open, soft structure, and are relatively unaffected by pH and temperature, enabling a range of applications such as eliminating odour, cleaning up oil spills, and slow release of absorbed water and dissolved nutrients in sports turf (www.bmpnz.co.nz). A preliminary field study on a commercial dairy farm showed that a natural zeolite feed additive can reduce plasma urea N in dairy cows and subsequent modelling indicated that this may lead to a reduction in urinary N excretion (K. Stelwagen, unpublished data).

It has been hypothesised that zeolites fed to ruminants have the potential to adsorb excess ammonium, and can release it as rumen ammonium concentrations decline (Mumpton & Fishman 1977; Bosi et al. 2002). Zeolites are able to adsorb and also release K^+, Mg^+, Ca^+, Na^+. Jorgensen and Theilgaard (2014) demonstrated the value of feeding synthetic sodium aluminium silicate to non-lactating cows to prevent clinical and subclinical parturient hypocalcaemia.

The main objective of this proof-of-concept study was to evaluate the effect of a single dose of zeolite deposited in the rumen before the main morning meal, on changes in rumen ammonium concentrations over time (hours) in dairy cows consuming fresh-cut ryegrass herbage. A secondary objective was to determine the potential effect of zeolite on plasma urea N, milk urea N, urinary N, and faecal N concentrations after feeding.

Materials and methods

Experimental design

The trial was approved by the Ruakura Animal Ethics committee (Approval 14025) and conducted at the DairyNZ Lye research farm in Hamilton, New Zealand, during November 2016. Twelve rumen-cannulated lactating

dairy cows (7.0±2.4 years; body condition score 4.0±0.2; live weight 498±47 kg and 108±28 days in milk) were allocated to two groups of six on the basis of calving date, live weight, genetic merit and body condition score. The cross-over experimental design comprised of two periods of four days each, with three days between the two periods enabling clearance of zeolite from the digestive tract. Each period consisted of three days of adaptation and one day of measurements. Cows were grazed on ryegrass-dominant pasture throughout, except for approximately 8 h on the two measurement days when they were housed and fed in the tie-stall barn.

Cows were milked twice daily at approximately 7 am and 3 pm. Milk yield was determined with in-line milk meters in the parlour and composition measured using a MilkoScan machine (Foss MilkoScan FT1). Following AM milking, cows in the zeolite treatment group received 300 g of BPM-Zeolite (DAB grade, 50-100 microns, Blue Pacific Minerals, Tokoroa, New Zealand) inserted through the rumen cannula and mixed immediately into the rumen contents. Cows in the control group were yarded with the zeolite group, but did not receive any zeolite. Afterwards, all cows were returned to a new break of pasture as a single group.

On the two measurement days before AM milking, spot samples of urine and faeces, a rumen liquor sample (collected by aspiration from an indwelling weighted sampling probe), and a blood sample (coccygeal venepuncture into K_3EDTA-coated vacutainers), were collected from each cow. The zeolite was inserted through the rumen cannula immediately after milking and all cows were housed for 8 h in a tie-stall barn where they were individually fed fresh-cut ryegrass-dominant pasture. Feed was ad libitum and water was freely available. The weight of pasture offered and refused by each cow was measured. Pasture samples were collected for determination of dry matter (DM), chemical, and botanical composition. Cows were milked at approximately 3 pm, then returned to graze.

On each measurement day, rumen liquor was sampled prior to milking (0 h) and 1, 2, 4, 6, and 8 h after the start of feeding. In conjunction with the 8 h rumen samples, spot samples were taken of urine and faeces as well as blood (similar as with the AM sample). Milk samples were collected at the PM milking of the measurement day, and the AM milking of the next day for milk urea N measurements.

Sample processing

The DM% of pasture offered to the cows in the tie-stall barn on the measurement days was determined by oven drying triplicate subsamples (150±5 g fresh weight) to a constant weight (48 h) at 95°C. Chemical composition of oven-dried samples were determined using near infrared spectroscopy by Massey University Nutrition Laboratory.

Immediately following the collection of the rumen liquor samples, the pH (EcoScan pH meter; Eutech Instruments, Singapore) was measured and a 6-ml sample of liquor from each cow was retained and acidified with 0.2 ml of 50% hydrochloric acid (HCl), mixed and centrifuged (1120 × *g*, 10 min, 4°C). The supernatants were stored at −20°C for determination of NH_4^+ concentration by colorimetry using phenol plus nitroprusside reagents (Weatherburn 1967).

Blood samples were placed on ice immediately following their collection and later centrifuged at 3000 × *g* for 10 min at 4°C. Plasma was aspirated and stored at −20°C for future analysis.

Milk urea N (PM and AM samples analysed separately) and plasma urea N concentrations were measured using the kinetic UV assay for urea/BUN package (Cobas®) in the Roche Modular Analyser (Gribbles Veterinary, Hamilton). Faecal and urine (after being acidified) samples were frozen (−20°C) until determination of total N using a combustion elemental analyser with thermal conductivity detection (Eurofins NZ Laboratory Services Limited, Auckland).

Statistical analysis

Intake, milk yield, and milk composition data were analysed using analysis of variance, with cross-over period and treatment included as fixed effects and cow as random effect. Results are presented as means and standard deviation and P-value for the effect of treatment. The rest of the data were analysed using mixed-models approach for repeated measures analysis of variance (Proc Mixed, SAS 9.3). Rumen NH_4^+, as well as urinary, faecal, and plasma urea N were analysed both as absolute values and as difference from pre-dose baseline. Milk urea N was

Table 1 Pasture intakes, milk yield and composition over the eight-day trial period (two periods of four days each, excluding the cross-over period), milk urea nitrogen (N) concentration, blood plasma urea N concentrations pre- and post- tie-stall feeding (7 am and 3 pm, respectively), and mean urinary and faecal N concentrations[#] of a treatment group of cows receiving a daily dose of 300g zeolite at 7 am versus a control group.

Parameter	Mean ± SD		
	Control	Zeolite	P -values
Pasture DMI between 7 am and 3 pm (kg)	9.1 ± 2.15	9.5 ± 2.84	0.773
Milk yield (kg/day)	19.5 ± 3.22	19.9 ± 3.28	0.132
Milk fat (%)	4.3 ± 0.53	4.4 ± 0.57	0.890
Milk protein (%)	3.4 ± 0.25	3.4 ± 0.19	0.909
Milk lactose (%)	4.9 ± 0.16	4.9 ± 0.15	0.850
Milk urea N (mmol/L)	3.92 ± 1.804	3.97 ± 1.769	0.924
Plasma urea N pre-feeding (mg/100 mL)	12.72 ± 1.901	13.09 ± 1.458	0.971
Plasma urea N post-feeding (mg/100 mL)	15.70 ± 2.017	14.56 ± 1.903	0.061
Urine N (g/100g)	0.63 ± 1.206	0.66 ± 0.114	0.399
Faecal N (g/100 g DM)	2.90 ± 0.186	2.76 ± 0.132	0.013

[#] Treatment did not affect concentrations before or after tie-stall feeding

analysed as absolute value. The models included time relative to start of treatment (7 am; 0 h on the measurement days), treatment, and their interaction, as well as cross-over period as fixed effects and cow as random effect. Pre-dose baseline results were included as covariate for the analyses of difference from pre-dose baseline. Results are presented as least-squares means and standard error of the difference (SED) or 95% confidence interval. Significance was declared if P<0.05.

Results

The pasture fed during measurement periods averaged 15.4±0.6% DM and the chemical composition (mean ± sd) was CP, 17.7±0.5; neutral detergent fibre (NDF) 44.3±2.0, ash 9.2±0.6 and lipid 3.3±0.3. Readily fermentable carbohydrate (100 – (percentage CP + NDF + ash + lipid)) averaged 25.6% of the DM whilst predicted metabolisable energy (MJ/kg DM) was 10.7±0.4 and organic matter digestibility was 78.6±4.3%. Dietary cation anion difference was 583.9±49.9 meq/kg DM.

The daily dose of zeolite did not affect intakes, milk yield or composition (Table 1). There was no overall effect of the zeolite treatment on rumen ammonium concentrations (P=0.29, Table 2). Rumen ammonium concentrations did increase after feed was offered (P=0.001), and although there was no treatment x time effect after feed was offered (P=0.49), the trend was for lower concentrations in Zeolite cows at 8 h (P=0.08, Table 2). The slower increase over time in rumen ammonium concentrations in the zeolite cows was evident from about 3 h after feeding, and the effect remained for the zeolite treated cows (Fig. 1).

Plasma urea N was similar for both treatments prefeeding and trended lower in Zeolite compared with Control cows in the measurements taken 8 h after the treatment (Table 1; P=0.06). However, the zeolite treatment had no effect on milk urea N or urinary N concentrations (Table 1) and the lower N concentration in faecal DM of cows given zeolite (P<0.05) was probably a consequence of dilution by undigested zeolite.

Figure 1 Change in rumen ammonium concentration (mmol/L) after the onset of feeding at approximately 7 am in two groups of cows, Zeolite and Control. Zeolite cows received a single dose of 300g zeolite immediately prior to feeding while Control cows received none. Least squared means and 95% confidence intervals are shown.

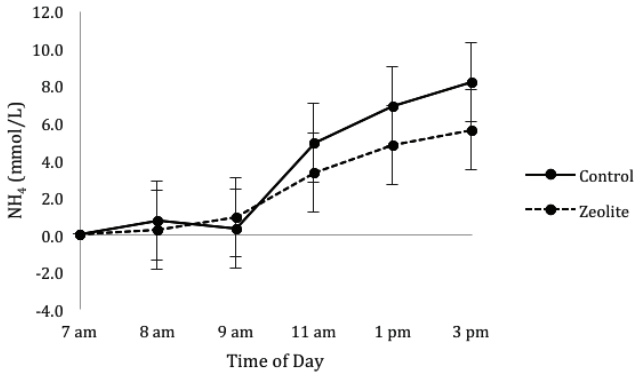

Table 2 Least-square means of ammonium concentrations (mmol/L) measured in rumen liquor samples taken from control and zeolite cows at different times of the day after a zeolite dose at approximately 7 am.

Time	Control	Zeolite	SED	P
7 am	5.01	4.91	1.431	0.947
8 am	5.51	4.99	1.431	0.721
9 am	5.05	5.67	1.431	0.666
11 am	9.70	8.06	1.431	0.257
1 pm	11.62	9.53	1.431	0.149
3 pm	12.91	10.33	1.448	0.080
Average	8.30	7.25	0.954	0.294

Discussion

The increase in rumen ammonium concentrations in both control and zeolite cows approximately 2 h after the onset of feeding is in agreement with other studies in which hourly measurements were taken from rumen-cannulated dairy cows (Hristov et al. 2004; Olijhoek et al. 2016). There was a consistent trend for rumen ammonium concentrations to increase at a slower rate in zeolite-treated cows from 3 h after dosing, with the difference between concentrations approaching significance at the 8-h sampling (P=0.08), indicating that zeolite was affecting ammonium concentrations. This trend of lower ammonium concentrations in zeolite-treated animals, suggests that the mean 24 h rumen ammonium concentrations could be reduced by zeolite treatment. A longer sampling duration after zeolite treatment, for example two-hourly measurements over 24 h, is required to confirm this hypothesis.

Lower rumen ammonium concentration corresponds with lower plasma urea N concentrations, and this may affect a reduction in urinary N excretion (Kebreab et al. 2001), especially if ammonium is bound to the zeolite and excreted in faeces.

However, despite this significant difference in plasma urea N concentrations, there were no effects on concentration of milk urea N, or urinary N measurements. This was unexpected since Gustafsson and Palmquist (1993) and Rodriguez et al. (1997) reported close associations between concentrations of milk and blood urea, with a 1- to 2- h lag between blood and milk urea peaks. In this study, milk urea concentrations (approximately 4 mmol/L; 24 mg /dL) were at the low end of the 'normal' range for mid-lactation dairy cows in New Zealand (DairyNZ 2017), probably because of the low dietary CP content (17.7%) compared with requirements of about 18% for lactating dairy cows (Pacheco & Waghorn 2008).

This study provides an indication that a daily dose of zeolite, fed prior to the morning meal, may have a moderating effect on rumen ammonium concentration, with a consequent reduction in plasma urea N concentrations of lactating dairy cows fed a ryegrass-dominant diet. However,

the effects of zeolite on rumen ammonium concentrations were small, without clear effects on digestion and milk composition, despite relatively low dietary CP.

Acknowledgements

This work was funded by the Pastoral 21 research programme which is a collaborative venture between the Ministry of Business, Innovation and Employment (MBIE), DairyNZ, Fonterra, Beef + Lamb NZ, PGG Wrightson Seeds and Blue Pacific Minerals Ltd. Thanks to the DairyNZ technical staff for handling the cannulated cows and the tie-stall work and thanks to Barbara Kuhn-Sherlock for advice on trial design and statistical analysis.

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