Altering the particle size of supplemental zeolite (clinoptilolite): effects on nitrogen utilization and nutrient digestibility in backgrounding cattle[1]

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INTRODUCTION

The indiscriminate breakdown of dietary nitrogen (N) by the ruminal microbes, and subsequent loss of ammonia-N (NH_3-N) from the rumen into blood, is the primary reason for the capture of only 10% to 20% of feed N into meat protein in beef cattle, with the remainder (80% to 90%) excreted as urine and fecal N (Satter et al., 2002). Because it is more environmentally labile than fecal N, excreted urine N, primarily urea-N (UUN), contributes to reactive N emissions (e.g., NH_3-N) that compromise ecological health. Thus, limiting UUN excretion has the potential to reduce the environmental cost of beef production.

Silicate minerals, including zeolite have a high ion exchange affinity for ammonium (NH_4^+) ions; White and Ohlrogge (1974) reported the binding of up to 15% of ruminal NH_4^+ by zeolites in both in vitro and in vivo studies. Thus, this could possibly explain the noted decrease in ruminal NH_3-N concentration at 6 and 9 h postfeeding in crossbred steers fed high concentrate diets containing 2.5% (dry matter [DM] basis) clinoptilolite (a naturally occurring zeolite; McCollum and Galyean, 1983). Sadeghi and Shawrang (2006) also reported a decrease in plasma urea-N (PUN) 3 h postfeeding in steers fed urea-N (2% of diet DM) in combination with clinoptilolite (3%) compared with just urea-N. However, on the other hand, feeding up to 3% supplemental clinoptilolite to finishing steers and lactating dairy cows did not result in a decrease in ruminal NH_3-N and milk urea-N concentrations (Dschaak et al., 2010; Urías-Estrada et al., 2018).

Besides purity, the particle size of zeolite can influence its impact on N metabolism (Papaioannou et al., 2005). For instance, in an in vitro study, the amount of NH_4^+ adsorbed over 24 h was 50% greater for clinoptilolite with a particle size distribution of 50 to 250 µm compared with 250 to 500 µm (Leung et al., 2006). Kotoulas et al. (2019) also reported a greater NH_4^+ adsorption rate as particle size decreases due to an increase in available surface area. However, in most feeding studies, the purity and particle size of zeolite used are not reported. To the best of our knowledge, the impact of feeding the same type of zeolite of the same purity, but with a different particle size on N utilization in cattle is yet to be evaluated. Thus, our objective was to assess the effects of feeding supplemental clinoptilolite with a particle size of either 30 or 400 µm (US 40 mesh) on ruminal NH_3-N and PUN concentrations, route of N excretion, and apparent total tract nutrient

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digestion in backgrounding cattle. We hypothesized that feeding supplemental clinoptilolite in backgrounding diets reduces the ruminal loss of NH_3-N, thus limiting UUN excretion, with particle size impacting the magnitude of the reduction in UUN excretion.

MATERIALS AND METHODS

All procedures in this study were approved by the Institutional Animal Care and Use Committee at the University of Idaho (Protocol # 2019-68).

Animals, Experimental Design, and Treatments

Six ruminally cannulated (10 cm diameter, Bar Diamond, Inc., Parma, ID) British crossbred beef heifers (initial body weight (BW) ± SD; 598 ± 55.6 kg) were used in a replicated 3 × 3 Latin square design with 21-d experimental periods. Sample collection was from d 15 to 21. Heifers were housed in individual tie-stalls (University of Idaho Beef Center) and fed at 1100 h daily. Dietary treatments were: 1) a typical forage-based basal backgrounding total mixed ration (TMR) with no supplement (CON), 2) CON + 30-µm clinoptilolite (CL-30), and 3) CON + 400-µm clinoptilolite (CL-400). The basal diet contained (% DM): hay, 25%; alfalfa silage, 35%; corn grain, 22.5%; distillers' grains, 15%; and mineral mix (2.5%), and had a crude protein (CP) content of 14.2%. Clinoptilolite (Ida-Ore Zeolite, Nampa, ID) was top-dressed (2.5% of diet DM) during morning feeding. The oxide content of clinoptilolite (90% purity) used was as follows (% DM): Si, 71.5%; Al, 11.3%; K, 4.55%; Fe, 2.05%; Na, 1.24%; Ca, 1.22%; and Ba, 0.15%).

Measurements

Heifers were weighed on two consecutive days at the beginning of each experimental period and at the end of the study. To determine dry matter intake, TMR offered, and orts were recorded daily. Feed ingredient and TMR samples were collected on three consecutive days each week and composited by week. Orts were collected daily and composited by animal and week. All samples were dried (55 °C; 72 h) and sequentially ground through a 4- and 2-mm screen (Retsch Cutting Mill SM 200, Retsch, Haan, Germany).

To measure fermentation characteristics, approximately 1 L of ruminal digesta from the cranial ventral, caudal ventral, central, and cranial dorsal regions of the rumen was collected 2, 3, 4, 6, and 12 h postfeeding on d 19. After straining through four layers of cheesecloth, a 5-mL aliquot was collected and mixed with 1 mL of 1% H_2SO_4 for later analysis of NH_3-N.

To determine apparent total tract nutrient digestibility and nutrient excretion, grab fecal and spot urine samples were collected on d 19 (1400 and 1900 h), d 20 (0300, 0700, 1500, and 2300 h), and d 21 (0500 and 1100 h). Collected fecal samples were immediately frozen (−20 °C). A 50-mL subsample of the collected urine was immediately acidified with 3 mL of 2 M H_2SO_4 to a pH < 2.5 and frozen (−20 °C) for later total N, urea-N, and creatinine analysis.

On the last day of each period (d 21) blood samples were collected 3 h postfeeding. Samples were centrifuged (645 × *g* for 25 minutes at 4 °C) immediately, with plasma frozen (−20 °C) for later PUN analysis.

Sample Analyses

Fecal samples were thawed overnight, composited by period, dried at 55 °C for 72 h, and sequentially ground through a 4- and 2-mm screen (Retsch Cutting Mill SM 200, Retsch, Haan, Germany). The ground TMR, orts, and fecal samples were then sent to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) for DM, organic matter (OM), neutral detergent fiber (NDF), and CP analysis. The indigestible NDF (iNDF) content of TMR and fecal samples was determined as described by Valente et al. (2011).

Ruminal fluid samples were centrifuged (10,800 × *g* for 20 minutes at 4 °C), with the supernatant analyzed for NH_3-N using a phenol–hypochlorite assay (Broderick and Kang, 1980). Urine samples were analyzed for total N using the Kjeldahl procedure (AOAC, 1990; method 976.05). Commercial kits (Arbor Assays, Ann Arbor, MI) were used for urine creatinine, UUN, and PUN analysis. Urine output was estimated using the concentration of creatinine measured in urine, and BW and creatinine constant of 29 mg/kg BW/d (Valadares et al., 1999). Fecal DM output was calculated by dividing iNDF intake (kg/d) by the fecal iNDF concentration.

Statistical Analysis

Nutrient intake, digestibility, and excretion data were analyzed using the MIXED procedure of SAS (SAS 9.4; SAS Inst. Inc., Cary, NC) for a replicated 3 × 3 Latin square. The model included

cow, period, square, and diet. Period, square, and diet were considered fixed, whereas cow within square was considered as random. Ruminal NH_3-N data were analyzed accounting for repeated measures through the inclusion of time (hour) and diet × time interaction in the model described previously. Data are presented as least square means. Significance was declared at $P < 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

There was no diet effect ($P = 0.50$) on ruminal NH_3-N concentration; however, concentration increased and peaked 3 h postfeeding before declining ($P < 0.01$; Fig. 1). Fecal, urine, and total N excretion did not differ ($P \geq 0.13$) across diets (Table 1). Similarly, PUN concentration at 3 h postfeeding did not differ ($P = 0.50$) across diets. There was no diet effect ($P \geq 0.11$) on DM, OM, and CP intake. Although it did not differ for the CL-400 compared with CON and CL-30 heifers, NDF intake tended ($P = 0.098$) to be lower for CL-30 that CON heifers. Similarly, although it did not differ for the CL-400 compared with CON and CL-30 heifers, apparent total tract OM digestibility was greater ($P = 0.03$) for CL-30 that CON heifers. However, there was no diet effect ($P \geq 0.26$) on apparent total tract DM, CP, and NDF digestibility.

DISCUSSION

Feeding supplemental clinoptilolite (30- and 400-µm) had no impact on ruminal NH_3-N concentration. We had anticipated a decrease in NH_3-N concentration as a result of the reported cation binding capacity of crystalline zeolites. Galyean and Chabot (1981) also did not observe changes in ruminal NH_3-N concentration after feeding clinoptilolite (3% of diet DM; 500 µm; purity not reported). Dschaak et al. (2010) made similar observations when feeding 1.4% (DM basis) clinoptilolite (particle size and purity not reported); since only a 4 h postfeeding sample was collected, this was attributed to the timing of sampling as NH_3-N sequestered following a meal is slowly released as ruminal concentration declines. In the present study, ruminal NH_3-N concentration did not differ at 2, 3, 4, 6, and 12 h postfeeding, with peak concentration at 3 h postfeeding. On the other hand, McCollum and Galyean (1983) observed a decrease in ruminal NH_3-N at 3, 6, and 9 h postfeeding when diets contained 2.5% to 5.0% clinoptilolite (<300 µm; 88% purity). Given the incomplete reporting of physicochemical properties, it is challenging to make across study comparisons. Since McCollum and Galyean (1983) noted changes when feeding a high concentrate diet compared to the high forage diet in the present study, and the study by Galyean and Chabot (1981), it is possible that besides physicochemical properties, diet associative effects could partially account for the discrepancies across studies. However, this needs to be further evaluated.

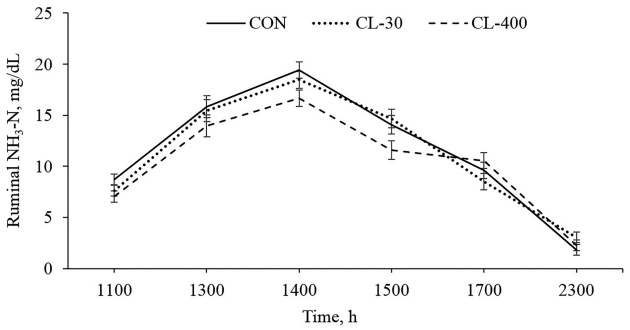

Figure 1. Rumen NH_3-N concentration for heifers fed a basal TMR with no supplement (CON), or supplemental 30- (CL-30) and 400-µm clinoptilolite (CL-400) at 2.5% of diet DM. Heifers were fed once daily at 1100 h. Diet, $P = 0.50$; Time, $P < 0.01$; Diet × Time interaction, $P = 0.77$. The error bars reflect the SEM associated with time.

Because feeding clinoptilolite had no impact on ruminal NH_3-N concentration, PUN concentration, and urine N and UUN excretion also did not differ across diets in the current study. Similarly, although PUN and UUN were not measured, milk urea-N concentration did not differ across diets as feeding 1.4% clinoptilolite (DM basis) also had no impact on ruminal NH_3-N concentration (Dschaak et al., 2010). On the other hand, PUN concentration was lower 3 h postfeeding in steers fed urea-N (2% of diet DM) in combination with clinoptilolite (3% DM) compared with urea-N alone (Sadeghi and Shawrang, 2006). Thus, increased binding of ruminal NH_3-N by clinoptilolite at a time when concentration increased could account for this observation. However, urine N and urea-N excretion were not measured in that study.

Although OM intake did not differ across diets, the greater apparent total tract OM digestibility for CL-30 compared with the CON steers resulted in OM excretion (data not shown) also being lower in the present study. Others (McCollum and Galyean, 1983; Dschaak et al., 2010) did not observe changes in DM or OM intake when feeding up to 5% (DM basis) clinoptilolite. On the other hand, although it had no impact on OM intake, ruminal OM digestibility increased (linear), whereas apparent total tract OM digestibility tended to increase as the clinoptilolite supplementation rate increased to 3% of diet DM, which was attributed to a tendency for an increase

Table 1. Nutrient intake, and digestibility, nitrogen excretion, and PUN concentration for heifers fed a basal TMR with no supplement (CON), or supplemental 30- (CL-30) and 400-µm clinoptilolite (CL-400) at 2.5% of diet DM

	Diet				
Item	CON	CL-30	CL-400	SEM	*P*-value
Nutrient intake, kg/d					
DM	16.1	15.2	15.8	1.00	0.11
OM	15.1	14.3	14.8	0.92	0.12
CP	2.29	2.17	2.25	0.140	0.12
NDF	6.00	5.66	5.88	0.376	0.098
Nutrient digestibility, % of intake					
DM	55.9	57.1	55.2	2.15	0.26
OM	57.8[a]	61.3[b]	60.0[ab]	2.04	0.03
CP	56.3	58.5	56.8	2.61	0.58
NDF	47.9	50.8	50.2	2.96	0.47
Fecal excretion					
N, g/d	160	144	156	13.5	0.28
N, % of N intake	43.7	41.5	43.2	2.61	0.58
Urinary excretion					
Total output, kg/d	12.6	11.2	11.4	2.48	0.70
N, g/d	171	151	157	21.3	0.46
N, % N intake	45.7	43.6	43.5	4.03	0.77
Urea-N, g/d	104	96	93	9.8	0.33
Urea-N, % of total urine	63.1	65.6	61.0	2.60	0.24
PUN, mg/dL	22.1	23.5	24.1	1.18	0.50

[ab]Means with different superscripts differ ($P < 0.05$).

in ruminal starch digestion (Urías-Estrada et al., 2018). Although not measured in the present study, ruminal starch digestibility could possibly have been greater for CL-30 than CON steers since total ruminal short-chain fatty acid (SCFA) concentration at 3 h postfeeding (data not shown) was also greater. In broiler chickens and pigs, feeding sepiolite improved OM digestibility by reducing digesta viscosity, which increased total tract retention time (Ouhida et al., 2010). Although shown to increase viscosity in vitro (Spotti et al., 2005), it is unlikely this occurs in vivo given the low inclusion levels used in most cattle studies and the large size of the gut. Moreover, ruminal volume and fluid dilution rate did not change when feeding up to 5% (DM basis) clinoptilolite (Galyean and Chabot, 1981; McCollum and Galyean, 1983). Thus, it is unlikely that supplemental clinoptilolite decreased digesta passage rate, possibly accounting for the greater apparent total tract OM digestibility for CL-30 than CON steers.

In summary, although feeding supplemental clinoptilolite with a particle size of either 30 or 400 µm to beef heifers had no impact on all measures of N utilization, apparent total tract OM digestibility was greater for heifers fed supplemental 30-µm but not 400-µm clinoptilolite compared with the CON diet.

Conflict of interest statement. None declared.

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