Effect of feeding ensiled or dried grape pomace on nitrogen utilization in backgrounding cattle

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INTRODUCTION

In beef cattle, excreted N nitrogen (N), which accounts for up to 90% of feed N, is converted to reactive N emissions including ammonia-N (NH3-N) and nitrates (NO3−) that compromise both air and water quality (Satter et al., 2002). Because it is more environmentally labile than fecal N, some of the effort to curb reactive N emissions has centered on dietary manipulation to reduce urinary N excretion (Waldrip et al., 2015).

Dietary inclusion of polyphenolic compounds including condensed tannins (CT) can favorably shift the route of N excretion from urine to feces in ruminants (Makkar, 2003). Polyphenolic compounds can cause a decrease in ruminal protein degradation by binding dietary protein, inhibiting the growth and activity of ruminal microbes, and suppressing the activity of microbial proteases (Vasta et al., 2019). This then could account for the observed decrease in the ruminal NH3-N concentration and, thus, urinary excretion of N and urea-N when beef cattle diets contained up to 4.5% tannin extracts (up to 3.5% CT on a dry matter [DM] basis; Koenig and Beauchemin, 2018; Norris et al., 2020). The irreversible binding of dietary protein by CT, which rendered it indigestible along the entire gastrointestinal tract, possibly explains the reported increase in fecal N excretion in those studies. However, the recommendation is for a maximum dietary inclusion level of 2% to 4% CT (DM basis) to prevent a detrimental decrease in nutrient supply and, thus, growth performance (Makkar, 2003). Although effective in altering N metabolism, it might not be economical to feed CT extracts to beef cattle. Therefore, the use of cheaper sources of polyphenolic compounds like grape pomace (GP) has greater appeal. In addition, GP also supplies other nutrients like lipids and crude protein (CP). Although information is still limited, there are indications that feeding GP can result in beneficial changes in N metabolism. For instance, Greenwood et al. (2012) reported a decrease in milk urea-N and plasma urea-N concentrations and increase in fecal N excretion in lactating cows fed fresh GP (17% of diet DM).

Because it has a high moisture content, the shelf-life of fresh GP is limited, which necessitates ensiling or drying to prevent spoilage. However, the preservation method used for forages and byproducts can lead to changes in the biological activity of polyphenolic compounds (Makkar and Singh, 1995). For instance, there was a decrease in the total CT content and soluble CT fraction, and an increase in the protein-bound CT fraction following the drying of safflower and birdsfoot trefoil (Girard et al., 2018). Moreover, drying GP led to a decrease in the total phenolics and tannin content (Taşeri et al., 2018). However, information on the potential impact of the preservation method used for GP on N metabolism is scarce. Therefore, our objective was to evaluate the effects of dietary inclusion of ensiled or sun-dried GP on ruminal NH3-N concentration, and the route of N...
We hypothesized that feeding GP as a source of CT impacts N metabolism in a manner that shifts N excretion from urine to feces, with effectiveness greater for ensiled than sun-dried GP.

### MATERIALS AND METHODS

All procedures in this study were approved by the Institutional Animal Care and Use Committee at the University of Idaho (Protocol # 2018-10).

**Animals, Experimental Design, and Treatments**

Six ruminally fistulated crossbred beef heifers (427 ± 27.0 kg; initial body weight [BW] ± SD) were used in a replicated 3 x 3 Latin square design with 21-d experimental periods. Sample collection was from days 19 to 21. Heifers were housed in individual tie-stalls (University of Idaho Dairy Center) and fed at 0700 h daily. Dietary treatments were as follows: 1) a typical backgrounding diet (CON), 2) CON + 15% ensiled GP (ENS), and 3) CON + 15% sun-dried GP (DRY). GP partially replaced triticale silage in the diet (Table 1). Diets were isonitrogenous (13% CP; DM basis) by design. Because its content did not differ in GP, dietary CT content was comparable between the ENS and DRY diets. Dietary addition of GP also resulted in an increase in acid detergent fiber (ADF), neutral detergent fiber (NDF), and indigestible NDF (iNDF) content.

### 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, total mixed ration (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 1-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 at 0, 1, 2, 3, 5, 8, 11, 14, 17, and 20 h postfeeding on day 19. After straining through polyester monofilament fabric (350-μm mesh opening; ELKO Filtering Co, LLC, Fort Lauderdale, FL), a 5-mL aliquot was collected and mixed with 1 mL of 1% H₂SO₄ for later analysis of NH₃-N.

To determine the route of N excretion, grab fecal and spot urine samples were collected on day 19 (0900, 1500, and 2100 h), day 20 (0300, 1200, and 1800 h), and day 21 (0000 and 0600 h). Collected

### Table 1. Dietary ingredient and chemical composition of experimental diets

| Item | CON | ENS | DRY |
|------|-----|-----|-----|
| Ingredient, % of DM | | | |
| Triticale silage | 54.3 | 40.8 | 40.8 |
| Grape pomace, ensiled | — | 15.0 | — |
| Grape pomace, dry | — | — | 15.0 |
| Corn grain, dry rolled | 30.0 | 30.0 | 30.0 |
| Canola meal | 14.5 | 13.0 | 13.0 |
| Performix mineral mix² | 1.16 | 1.16 | 1.16 |
| Chemical composition | | | |
| DM | 64.6 ± 3.67 | 62.7 ± 2.47 | 71.2 ± 3.00 |
| OM, % of DM | 93.2 ± 0.07 | 91.1 ± 0.38 | 92.9 ± 0.20 |
| CP, % of DM | 13.0 ± 0.25 | 13.1 ± 0.30 | 13.0 ± 0.25 |
| ADF, % of DM | 22.9 ± 0.46 | 27.5 ± 0.58 | 26.7 ± 0.38 |
| NDF, % of DM | 39.0 ± 0.91 | 41.5 ± 0.98 | 40.6 ± 0.69 |
| iNDF, % of DM | 16.9 ± 0.71 | 20.7 ± 0.11 | 20.3 ± 0.62 |
| Tannins³, % | — | 0.50 ± 0.022 | 0.50 ± 0.072 |

¹CON (control), ENS (ensiled grape pomace), DRY (dried grape pomace).
²Supplement DM contained CP, 51.3%; crude fat, 0.48%; Salt, 12.3%; Ca, 19.7%; P, 0.07%; Mg, 0.55%; K, 0.10%; S, 0.15%; Fe, 12.5%; Mn, 1.230 mg/kg; Zn, 2.050 mg/kg; organic Zn, 1,025 mg/kg; Cu, 615 mg/kg; organic Cu, 205 mg/kg; Co, 31.2 mg/kg; I, 175 mg/kg; Se, 13.5 mg/kg; Selenium yeast, 4.51 mg/kg; Vitamin A, 27,948 IU/kg; Vitamin D, 2,795 IU/kg; Vitamin E, 93.1 IU/kg; and Rumensin 90 (Elanco Animal Health, Greenfield, IN), 1,128 g/ton.
³Calculated based on the tannin content of the GP used in the present study (average of 3.33% and 3.53% DM for ensiled and sun-dried GP).
fecal samples were immediately frozen (−20 °C). A 50-mL subsample of the collected urine was immediately acidified with 3 mL of 2 M H₂SO₄ to a pH < 2.5 and frozen (−20 °C) for later total N, urea-N, and creatinine 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 1-mm screen (Retsch Cutting Mill SM 200, Retsch, Haan, Germany). The ground TMR, orts, and fecal samples were analyzed for DM (AOAC, 2005; method 930.15), organic matter (AOAC, 2005; method 942.05), ADF and NDF (AOAC, 2005; method 2002.04), and CP (AOAC, 1990; method 976.05). The iNDF content of TMR and fecal samples was determined as described by Valente et al. (2011). GP samples were also analyzed for CTs as described by Khiaosa-Ard et al. (2015).

Ruminal fluid samples were centrifuged (10,800 × g for 20 min at 4 °C), with the supernatant analyzed for NH₃-N using a phenol-hypochlorite assay (Broderick and Kang, 1980). Urine samples were analyzed for total N (AOAC, 1990; method 976.05). Commercial kits (Arbor Assays, Ann Arbor, MI) were used for urine creatinine, and urea-N (UUN) analysis. Urine output was estimated using the concentration of creatinine measured in urine, and BW and creatinine constant of 29 mg/kg BW per day (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 NH₃-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 ($P \geq 0.48$) on DM and N intake (Table 2). However, feeding GP resulted

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**Table 2.** Nutrient intake, and digestibility, nitrogen excretion, and plasma urea-N concentration for heifers fed a typical backgrounding diet (CON), or backgrounding diets containing either 15% ensiled (ENS) or 15% sun-dried grape pomace (DM basis)

| Item | CON | ENS | DRY | SEM | $P$-value |
|------|-----|-----|-----|-----|-----------|
| **Intake** | | | | | |
| DM, kg/d | 16.3 | 16.4 | 17.2 | 1.08 | 0.52 |
| N, g/d | 339 | 343 | 359 | 20.9 | 0.48 |
| **Fecal excretion** | | | | | |
| DM, kg/d | 6.27 | 6.99 | 7.00 | 0.588 | 0.22 |
| N, g/d | 122 | 145 | 144 | 8.1 | 0.02 |
| N, % of N intake | 36.1 | 42.4 | 40.0 | 1.29 | 0.01 |
| ATTND₁, % of intake | 63.9 | 57.6 | 60.0 | 1.292 | 0.01 |
| **Urinary excretion** | | | | | |
| Total output, kg/d | 8.71 | 8.41 | 6.40 | 1.286 | 0.30 |
| N, g/d | 126.6 | 90.3 | 86.5 | 7.45 | <0.01 |
| Urea-N, g/d | 84.9 | 67.1 | 62.1 | 6.05 | <0.01 |
| Urea-N, % of total urine intake | 68.6 | 74.1 | 69.8 | 2.61 | 0.15 |
| Total N, % N intake | 38.3 | 25.8 | 23.9 | 2.72 | <0.01 |
| **Total N excretion** | | | | | |
| g/d | 245 | 241 | 233 | 14.4 | 0.60 |
| % N of intake | 73.6 | 69.1 | 64.3 | 3.29 | 0.15 |
| Apparent N retention, g/d | 95.7 | 107.5 | 128.1 | 17.30 | 0.37 |
| Rumen NH₃-N², mg/dL | 7.99 | 6.88 | 6.76 | 0.386 | 0.04 |

¹Apparent total tract nitrogen digestibility.
²Time effect, $P < 0.01$; diet × time interaction, $P = 0.74$.
³Means for diet effect with different superscripts differ ($P < 0.05$).
in a decrease ($P = 0.04$) in ruminal NH$_3$-N concentration. Urine N (g/d and % of N intake) and urea-N (g/d) output were also lower ($P < 0.01$) for heifers fed GP-containing diets than the CON diet. Because apparent total tract N digestion was lower ($P = 0.01$), fecal N excretion (g/d and % of N intake) was greater ($P < 0.02$) for GP than CON heifers. However, there was no diet effect ($P \geq 0.15$) on total N excretion and apparent retention.

**DISCUSSION**

In the present study, feeding GP resulted in a decrease in ruminal NH$_3$-N concentration. Although not measured, this possibly was due to a tannin-induced decrease in ruminal protein degradation, since apparent total tract CP digestibility was also lower for ENS and DRY compared to CON heifers. This decrease in ruminal degradation has been reported to occur as a result of the binding of dietary protein and ruminal microbial proteases, and suppression of microbial growth and activity by tannins (Vasta et al., 2019).

One of the factors that could affect the efficacy of tannins in GP in modulating N metabolism is the preservation method used for feedstuffs. For instance, increasing the temperature (either freeze drying or drying at 50 and 100 °C) and duration (2, 4, 24, and 48 h) of drying for tannin-protein complexes resulted in an increase in fiber-bound CT (Makkar and Singh, 1995). Similarly, others (Girard et al., 2018; Taşeri et al., 2018) also reported a decrease in the total CT content and soluble CT fraction, and an increase in the protein-bound CT fraction following the drying of tannin-containing forages and GP. In the present study, both ensiled and sun-dried GP resulted in a comparable decrease in ruminal NH$_3$-N concentration. Although not reported, this possibly was due to the total tannin, and soluble and bound fractions being similar across products.

Feeding ensiled and sun-dried GP resulted in a decrease in urinary excretion of N and urea-N. This was expected given the decrease in ruminal N metabolism. Although it was not measured, Greenwood et al. (2012) reported a decrease in the plasma and milk concentration of urea-N when feeding fresh GP (17% of diet DM), which was suggestive of changes in ruminal N metabolism. However, although urine NH$_3$-N concentration decreased, there was no change in UUN concentration when feeding GP in that study. Urine urea-N, which makes up 56 to 93% of urine N in cattle (Bristow et al., 1992), is rapidly converted to reactive forms of N including NH$_3$-N and NO$_3^-$ that compromise both air and water quality (Hristov et al., 2011). Therefore, the decrease in UUN excretion in the present study is beneficial from an environmental standpoint. Feeding GP also led to an increase in fecal N excretion in the present study. This suggests the irreversible binding of dietary N by tannins along the gastrointestinal tract. Others (Greenwood et al., 2012; Vinyard et al., 2021) also made similar observations following dietary inclusion of 15 to 30% DM fresh and dried GP. Because its rate of mineralization is much slower than urine N, the shift to fecal N excretion is beneficial as it limits reactive N emissions and increases the value of manure as a fertilizer for crop production.

In summary, feeding GP irrespective of preservation method was effective in changing the route of N excretion from urine to feces, which is beneficial from an environmental standpoint.

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