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# *Technical note:* Effects of forage protein-binding polyphenols on chemistry of dairy excreta

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## ABSTRACT

Forage chemistry can affect intake, digestion, milk production, and manure excretion. Although information is available on the effects of forage protein-binding polyphenols on small ruminant production and manure excretion, little information is available for dairy cattle. The objective of this study was to compare fecal and urinary N excretion of diets formulated with alfalfa (*Medicago sativa* L.) silage versus condensed tannin-containing birdsfoot trefoil (*Lotus corniculatus*) or *o*-quinone-containing red clover (*Trifolium pratense* L.) silages. Significantly higher concentrations of N were excreted in urine by lactating Holstein dairy cows fed red clover and low-tannin birdsfoot trefoil (8.2 g/L) than by cows fed high-tannin birdsfoot trefoil or alfalfa (7.1 g/L). Fecal N concentrations were similar (33.6 g/kg) among all diets. Dairy cows fed red clover had lower rates of urinary N excretion (5.0 g/h) compared with other forages (6.6 g/h). Fecal N excretion rates were lowest for red clover (4.1 g/h), intermediate for alfalfa (5.8 g/h), and greatest for cows fed high- and low-tannin birdsfoot trefoil (6.4 g/h). The ratio of fecal N to urinary N was highest for high-tannin trefoil, lowest for alfalfa and red clover, and higher in excreta collected in morning than evening. Concentrations of neutral detergent fiber (NDF) in feces, of N in NDF (NDIN) and acid detergent fiber (ADIN), and relative amounts of NDIN and ADIN excreted in feces were significantly higher from cows fed high-tannin birdsfoot trefoil than the other silage types. Study results imply that collection of excreta for environmental studies needs to consider forage polyphenol and diurnal effects on chemistry of dairy excreta.
**Key words:** forage tannins, manure chemistry, feces, urine

## INTRODUCTION

Recent environmental concerns related to livestock contributions to greenhouse gas emissions and global warming (FAO, 2006) require new information on the relationships between livestock diets, production, excretion of C and N in manure, and the overall impact of dietary decisions and livestock management on atmospheric and terrestrial C and N cycles. The type and amount of CP consumed by dairy cattle affect total N excretions and the relative amount of N excreted in feces and urine. The type and amount of CP and forage consumed by dairy cattle affect volatile N losses (Misselbrook et al., 2005; Powell et al., 2008) and the transformation and plant uptake of manure N after application to soils (Sørensen et al., 2003; Powell et al., 2006). Modest amounts of condensed tannins (2 to 4% of DM), as are found in birdsfoot trefoil, reduce protein breakdown during ensiling and rumen fermentation by up to 50% (Albrecht and Muck, 1991; Broderick and Albrecht, 1997). In a New Zealand study, tannins in birdsfoot trefoil increased milk production of Holstein cows by 2.7 kg/d (Woodward et al., 1999). Experiments with sheep suggest that, in addition to enhancing protein utilization, tannins and polyphenols shift N excretion from urine to feces and from soluble to insoluble N forms in feces (Powell et al., 1994; Waghorn, 2008). Consumed forage tannins decrease urinary N excretion by lactating dairy cows and volatile N losses after land application of dairy manure (Misselbrook et al., 2005). Other forages such as red clover contain relatively high levels of *o*-quinones formed by polyphenol oxidase and *o*-diphenols, which limit proteolysis during forage protein conservation and ruminal fermentation (Broderick and Albrecht, 1997; Sullivan and Hatfield, 2006; Grabber, 2008).

The digestion of forages fed to ruminant livestock and the decomposition of organic materials applied to soils are governed by similar chemical and microbial processes (Chesson, 2002). Rumen and soil microorganisms readily digest soluble compounds (e.g., cytoplasmic proteins and carbohydrates) followed by recalcitrant plant fibers. Perhaps the most widely recognized assay to delineate readily digestible and recalcitrant fractions is the detergent system of fiber analysis (Van Soest and Robertson, 1980).

Relationships between excreted fecal fiber and consumed forage fiber have been reported for small rumi-

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**Table 1.** Ingredients and chemical composition of diets fed to lactating dairy cows[1]

| | Trial 1 | | | | Trial 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Ingredients, % of DM | ALF | LTBT | HTBT | RCL | ALF | LTBT | MTBT | HTBT | MIX |
| ALF silage | 51.2 | 0.0 | 0.0 | 0.0 | 47.9 | 16.0 | 16.0 | 16.0 | 0.0 |
| Corn silage | 10.4 | 10.4 | 10.3 | 10.1 | 18.0 | 18.0 | 18.0 | 18.0 | 18.0 |
| LTBT silage | 0.0 | 51.1 | 0.0 | 0.0 | 0.0 | 32.0 | 0.0 | 0.0 | 16.0 |
| MTBT silage | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 32.1 | 0.0 | 16.1 |
| HTBT silage | 0.0 | 0.0 | 51.1 | 0.0 | 0.0 | 0.0 | 0.0 | 32.0 | 16.0 |
| RCL silage | 0.0 | 0.0 | 0.0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Rolled high-moisture shell corn | 32.6 | 30.9 | 29.0 | 30.0 | 23.8 | 23.8 | 23.8 | 23.8 | 23.8 |
| Solvent-extracted soybean meal | 5.1 | 6.9 | 8.9 | 9.3 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 |
| Soyhulls | 0.0 | 0.0 | 0.0 | 0.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| EN Booster[2] | 0.0 | 0.0 | 0.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Dicalcium phosphate | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Sodium bicarbonate | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Salt | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Vitamin/mineral premix | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| CP, % of DM | 19.3 | 16.9 | 15.7 | 15.8 | 17.6 | 16.5 | 16.8 | 16.9 | 16.4 |
| Tannin,[3] % of DM | ND[4] | 0.56 | 1.66 | 0.08 | ND | 0.51 | 0.84 | 1.48 | 0.94 |

[1]Diets were formulated by replacing silage from alfalfa (ALF) with silage from low-tannin birdsfoot trefoil (LTBT), high-tannin birdsfoot trefoil (HTBT), or *o*-quinone-containing red clover (RCL). In trial 2, MTBT = medium-tannin birdsfoot trefoil, and MIX comprised equal portions of corn silage, LTBT, MTBT, and HTBT silages.

[2]Energy Booster 100; Milk Specialties (Dundee, IL).

[3]Refers to tannin concentration only in the LTBT, MTFT, HTBF, and RCL diet components.

[4]ND = not detectable using the butanol-HCl assay (Grabber, 2008).

nants (Somda et al., 1995; Somda and Powell, 1998), and for dairy cattle (Sørensen, 2003; Powell et al., 2006). Ruminant feces, being the principal by-product of forage degradation by rumen microorganisms, have much higher levels of fiber N than the forages from which the feces were derived (Somda et al., 1995; Somda and Powell, 1998). The N contained in ruminant feces consists of 1) endogenous N from microorganisms or microbial products from the rumen, small intestine, and hindgut, and N originating from the digestive tract itself; and 2) undigested fiber N (Mason and Frederiksen, 1979). After application to soil, fecal endogenous N mineralizes quickly, but fecal undigested fiber N degrades much more slowly (Sørensen et al., 1994; Somda et al., 1995; Powell et al., 1999). After application to soil, microbial mineralization of N contained in sheep feces (Powell et al., 1999) and dairy manure (Sørensen et al., 2003) was negatively related to dietary NDF.

The collection of dairy excreta for use in environmental studies can be costly and labor intensive. Our previous work involved short-term collection of small amounts of excreta for laboratory studies (Misselbrook et al., 2005; Powell et al., 2006). Longer term collections may be required to collect sufficient excreta for large field-scale studies. There is a lack of information on how diet affects diurnal variation in the chemical composition of dairy excreta, and how the excreta affect soil C and N cycles. The objectives of the present study were 1) to determine the effect of protein-binding tannins and *o*-quinones in forages on the diurnal pattern of fecal and urinary N concentration and excretion; and 2) to assess the effect of forage tannin content on fecal fiber fractions and their N concentrations.

## MATERIALS AND METHODS

Feces and urine were collected separately during a trial (trial 1; U. Hymes-Fecht, unpublished data; Table 1) designed to assess effects on milk yield and N utilization by substituting silage from alfalfa (**ALF**) with silage from low-tannin birdsfoot trefoil (**LTBT**), high-tannin birdsfoot trefoil (**HTBT**), or *o*-quinone-containing red clover (**RCL**). Diets were evaluated for a period of 4 wk using a 4 × 4 Latin square switchback design. At the end of each 4-wk diet evaluation period, before cows were reassigned to their next diet, indwelling catheters were inserted into 3 randomly selected cows per diet and total fecal and urine excretions were collected over the next 72 h. Feces were hand-scraped from metal catchment containers fitted into barn gutters, and urine was collected by draining catheters into plastic carboys embedded in ice. Two collections per day were done, which coincided with the 30-min periods when cows were absent from tie-stalls during twice-daily milking. Catheter tubing to collection pails was detached (Keck tubing adapters, Cole Parmer Instrument Co., Vernon Hills, IL) and tubing ends protruding from cows were clipped shut to avoid urine loss during milking. Observations indicated minimal excretion of feces during the period when cows were away

**Table 2.** Trial 1 ANOVA for forage type and excreta collection period effects on fecal N (FN), urinary N (UN), FN:UN excretion ratio, and excretion rates of FN (ExFN) and UN (ExUN)

| | | FN (g/kg of DM) | | UN (g/L) | | FN:UN (g/g) | | ExFN (g/h) | | ExUN (g/h) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | df | *P*-value | % Trt.[1] | *P*-value | % Trt. | *P*-value | % Trt. | *P*-value | % Trt. | *P*-value | % Trt. |
| Forage type[2] (F) | 3 | 0.400 | — | <0.0001 | 99.4 | 0.0087 | 48.3 | <0.0001 | 49.3 | 0.005 | 54.2 |
| Period[3] (P) | 1 | 0.609 | — | 0.876 | — | 0.0027 | 36.8 | <0.0001 | 48.9 | 0.0008 | 34.2 |
| F × P | 3 | 0.601 | — | 0.585 | — | 0.348 | — | 0.884 | — | 0.387 | — |

[1]Percentage of total treatment variation attributable to F, P, and F × P individual effect.

[2]Diets were formulated by replacing silage from alfalfa with silage from low-tannin birdsfoot trefoil, high-tannin birdsfoot trefoil, or *o*-quinone-containing red clover.

[3]AM collection period comprised excreta from approximately 0400 to 1500 h (11-h period); PM collection period comprised excreta from approximately 1500 to 0400 h (13-h period).

from stalls during milking. Feces and urine from 0400 to 1500 h (i.e., 11 h) comprised the morning (**AM**) collection period, and excreta from 1500 to 0400 (i.e., 13 h) comprised the afternoon (**PM**) collection period. To allow cows to adapt to catheters, feces and urine were discarded during the first AM collection period. Feces and urine were weighed and subsamples were frozen immediately until analyses.

Feces only were collected at the end of another trial (trial 2; U. Hymes-Fecht, unpublished data; Table 1) that evaluated effects on milk yield and N utilization of feeding second-cut ALF, LTBT, medium-tannin birdsfoot trefoil (**MTBT**), HTBT silages, or a diet (**MIX**) comprising equal portions of corn silage, LTBT, MTBT, and HTBT silages. Within 2 h after cows returned to tie-stalls after the trial's last milking, single fecal samples were taken directly from the rectum of 8 cows per diet and frozen immediately until analyses.

Feces derived from all diets (Table 1) were thawed, oven-dried (60°C) to a constant weight, and ground to pass a 1-mm sieve. Subsamples were dried at 100°C for 24 h for DM determination. Total N contained in feces and urine was determined on a VarioMax Elementar Analyzer (Hanau, Germany). Cell wall components of feces were determined using the modified detergent system (Van Soest and Robertson, 1980) as NDF and ADF. Total N contained in NDF (**NDIN**) and ADF (ADIN) was also determined on the VarioMax. Subsamples of LTBT, MTBT, HTBT, and RCL were taken before feeding, quick frozen in liquid N, freeze-dried, ground to pass a 1-mm sieve, and then analyzed in triplicate for total soluble plus insoluble condensed tannins using a butanol-HCl assay (Grabber, 2008). Effects of diet on excreta properties were analyzed by generalized least squares ANOVA (SAS Institute, 1990). When relevant, the protected least significant difference (LSD) test was used to determine significant differences among treatments at $P < 0.05$.

## RESULTS AND DISCUSSION

For feces and urine collected from the first trial, there were no significant interactions of forage type and excreta collection period for any of the measured excreta

**Table 3.** Trial 1 forage type and excreta collection period effects on fecal N (FN), urinary N (UN), FN:UN excretion ratio, and excretion rates of FN (ExFN) and UN (ExUN)

| | Excreta property | | | | |
|---|---|---|---|---|---|
| Variable | FN (g/kg) | UN (g/kg) | FN:UN (g/g) | ExFN (g/h) | ExUN (g/h) |
| Forage[1] | | | | | |
| ALF | 33.0 | 7.0[b] | 44.7: 55.3[bc] | 5.8[b] | 6.8[a] |
| LTBT | 33.2 | 8.0[a] | 47.8: 52.2[ab] | 6.3[a] | 6.8[a] |
| HTBT | 33.9 | 7.2[b] | 50.7: 49.3[a] | 6.4[a] | 6.2[a] |
| RCL | 34.3 | 8.3[a] | 42.6: 57.4[c] | 4.1[c] | 5.0[b] |
| SE | 0.59 | 0.15 | 1.86 | 0.35 | 0.33 |
| Period[2] | | | | | |
| AM | 33.7 | 7.6 | 49.3: 50.7[a] | 6.6[a] | 6.7[a] |
| PM | 33.4 | 7.6 | 43.8: 56.2[b] | 4.7[b] | 5.6[b] |
| SE | 0.41 | 0.12 | 1.32 | 0.25 | 0.24 |

[a–c]Within a forage or period, column means followed by a different superscript letter differ significantly ($P <$ 0.05).

[1]Diets were formulated by replacing silage from alfalfa (ALF) with silage from low-tannin birdsfoot trefoil (LTBT), high-tannin birdsfoot trefoil (HTBT), or *o*-quinone-containing red clover (RCL).

[2]AM collection period comprised excreta from approximately 0400 to 1500 h (11-h period); PM collection period comprised excreta from approximately 1500 to 0400 h (13-h period).

**Table 4.** Trial 2 forage type effects on fecal N (FN), NDF, N concentrations in NDF (NDIN), ADF, N concentrations in ADF (ADIN), and percentage of FN contained in NDF (NDIN:FN) and ADF (ADIN:FN)

| | Fecal chemical property | | | | | | |
|---|---|---|---|---|---|---|---|
| Diet[1] | FN (g/kg) | NDF (g/kg) | NDIN (g/kg) | ADF (g/kg) | ADIN (g/kg) | NDIN:FN (g/g) | ADIN:FN (g/g) |
| ALF | 31.7 | 485[b] | 8.6[b] | 309 | 2.3[d] | 13.3[b] | 2.3[b] |
| LTBT | 31.5 | 488[ab] | 8.5[b] | 297 | 2.8[cd] | 13.2[b] | 2.6[b] |
| MBFT | 30.6 | 482[b] | 7.1[b] | 289 | 2.9[c] | 11.2[b] | 2.8[b] |
| HTBT | 33.3 | 516[a] | 11.9[a] | 307 | 3.8[b] | 18.6[a] | 3.5[a] |
| MIX | 34.1 | 505[ab] | 11.1[a] | 307 | 4.4[a] | 16.5[a] | 4.0[a] |
| SE | 0.58 | 47 | 0.40 | 41 | 0.12 | 0.62 | 0.13 |

[a–d]Column means followed by a different superscript letter differ significantly ($P < 0.05$).

[1]Diets were formulated by replacing silage from alfalfa (ALF) with silage from low-tannin birdsfoot trefoil (LTBT), medium-tannin birdsfoot trefoil (MTBT), or high-tannin birdsfoot trefoil (HTBT). In trial 2, MIX comprised equal portions of corn silage, LTBT, MTBT, and HTBT silages.

properties (Table 2). The type of forage consumed by dairy cows influenced urinary N (**UN**) concentrations and UN excretion rate. Forage type did not alter fecal N (**FN**) concentrations, but did alter FN excretion rate and FN:UN excretion ratios. Collection period did not affect FN and UN concentrations, but did significantly affect FN:UN excretion ratios and FN and UN excretion rates.

Fecal N concentrations were similar among diets (Table 3). Urine N concentrations were significantly higher in cows fed LTBT and RCL than in cows fed ALF or HTBT. The ratio of N excreted in feces and urine was greatest for LTBT and HTBT and lowest for ALF and RCL. Fecal N excretion rates were greatest for cows fed LTBT and HTBT, intermediate for ALF, and lowest for RCL. These results correspond with our previous findings (Misselbrook et al., 2005) that 1) ALF and HTBT resulted in similar UN concentrations, which were lower than UN from cows fed LTBT; 2) HTBT resulted in the highest FN:UN excretion ratio; and 3) forage tannin increased FN.

Significantly greater FN and UN excretion rates and greater FN:UN ratios were calculated from excreta collected in the morning compared with that collected in the evening (Table 3). These results contrast with other findings (Aland et al., 2002) that fecal and urinary excretions by housed dairy cattle are lower during rest periods (overnight excretions collected the following morning in the present study) than during active periods. Similar diurnal patterns in FN and UN concentrations across a range of forage diets imply that representative samples of feces and urine could be collected from daytime or nighttime excreta. If a representative FN:UN ratio is required, however, then collection over a longer period would be required to account for diurnal differences in FN and UN excretion rates.

In the second study, the forage component of the diet also affected fecal fiber chemistry (Table 4). In general, concentrations of NDF, NDIN, and ADIN, and NDIN:FN and ADIN:FN ratios (fractional amounts of NDIN and ADIN in total FN) were significantly higher in feces from cows fed HTBT than in that from cows fed the other forages. Forage type did not significantly affect concentrations of FN or ADF in dairy feces. These results and a summary of other trial results (Waghorn, 2008) demonstrate the effect of tannins on fecal and urinary N excretions by ruminants and imply that the type of forage consumed by dairy cattle affects fecal fiber composition (Powell et al., 2006) as well as fecal C and N cycling in soils (Somda et al., 1995; Somda and Powell, 1998; Powell et al., 2006).

## CONCLUSIONS

Data from this and other recent studies show that the type of forage consumed by dairy cows affects the chemistry of excreta, including concentrations of N in feces and urine, N excretion rates and ratios, concentrations of fiber fractions in feces, and the relative partitioning of N into fecal fiber fractions. With increasing scrutiny of livestock effects on greenhouse gas emission, soil carbon sequestration, and water and air quality, forage selection criteria may need to expand the current focus on dairy cattle health and milk production to also consider forage effects on manure chemistry and subsequent effects on the environment.

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