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Research Note

Condensed Tannin in Drinking Water Reduces Greenhouse Gas Precursor Urea in Sheep and Cattle Urine

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Abstract

Ingestion of small amounts of condensed tannin (CT) by ruminants may provide benefits including reduction of ammonia and nitrous oxide emissions by reducing urine urea excretion. However, providing grazing ruminants with sufficient amounts of CT-containing forages is difficult, and an alternative may be to provide CT in their drinking water. We conducted three trials to determine if urine urea levels in sheep and cattle decrease after they drink water containing CT. In two initial trials, blood serum urea was measured as a surrogate for urine urea when lambs or steers drank tap water containing low to higher amounts of quebracho tannin (QT). Serum urea concentration was measured after lambs drank the treatments for 7 d or steers for 4–6 d. Lambs consumed pellets (16% crude protein [CP] as fed) at 3.5% of body weight, and steers were fed cubes (15% CP as fed) at 3% of body weight. Mean serum urea concentration in sheep was reduced when they consumed water with QT ($P = 0.03$) and was also reduced for cattle ($P < 0.001$). In a third trial with a Latin-square design, four wethers were fed pellets (22% CP, DM basis) and given tap water or tap water with low, medium, or high amounts of QT, and their urine urea excretion was measured. There was a linear effect of QT intake on daily urine urea excretion as a percentage of nitrogen intake ($P = 0.03$). Reductions in daily urea excretion as a percentage of nitrogen intake were 3.5%, 6.6%, and 12.6%, respectively, for the low, medium, and high QT intake. Small amounts of QT in the drinking water of grazing ruminants can reduce their urine urea excretion.

Resumen

La ingestión de pequeñas cantidades de taninos concentrados (TC) por los rumiantes podría traer beneficios incluyendo, la reducción de emisiones de amonia y oxido nitroso al reducir la excreción de urea por la orina. Sin embargo, proveer a los rumiantes en pastoreo con suficiente cantidad de TC en el forraje es difícil y una alternativa podría ser agregar el TC en los abrevaderos. Realizamos tres pruebas para determinar sí el nivel de urea en la orina en ovinos y bovinos disminuía después que estos animales bebieran agua con TC. En dos pruebas iniciales, se medio suero sanguino con urea como sustituto de orina de corderos y novillos que bebieron agua conteniendo baja y alta cantidad de tanino de quebracho (TQ). Se midió el suero de urea concentrado después de que los corderos bebieron del tratamiento por 7 días y los novillos de 4 a 6 días. Los corderos consumieron el 3.5% de su peso vivo de suplemento en forma de pellets (16% de PC) y los novillos suplemento en forma de cubos (15% de PC) en cantidad correspondiente al 3% de su peso vivo. La media de la concentración de suero de urea en borregas se redujo cuando consumieron agua con TQ ($P = 0.03$), y también se redujo en bovinos ($P < 0.001$). En una tercera prueba con diseño de Cuadro Latino, cuatro corderos se alimentaron con pellets (22% PC, en base a materia seca) y tuvieron acceso a agua sin TQ o agua con cantidades bajo, media y alto de TQ y se medio las excreciones de urea en la orina. Hubo un efecto linear en el consumo de TQ y las excreciones diarias de urea en la orina como un porcentaje del consumo de nitrógeno ($P = 0.03$). La reducción de excreciones diarias de urea en la orina como porcentaje de consume de nitrógeno fueron 3.5%, 6.6%, y 12.6% respectivamente para el bajo, medio y alto consumo de TQ. Cantidades pequeñas de TQ en el agua de beber para ovinos en pastoreo puede reducir la excreción de urea en la orina.

Key Words: ammonia, greenhouse gas reduction, nitrous oxide, ruminants

INTRODUCTION

Increasing levels of nitrogenous compounds in the environment are associated with environmental and human health concerns (Vitousek et al. 1997; Townsend et al. 2003). Urea in ruminant urine is an important precursor of the nitrogenous pollutant ammonia and the potent greenhouse gas nitrous oxide (Thomas et al. 1988). Urine urea excreted on soil can be transformed by soil microorganisms into nitrous oxide and ammonia gases, which can then be emitted from soil to the atmosphere. Moreover, when ammonia returns to the soil in gaseous form or with precipitation, it can be transformed into nitrous oxide, thereby acting as an indirect greenhouse gas (Mosier et al. 1998). Bacterial decomposition of urea in animal urine is the largest global source of ammonia emissions (Dentener and Crutzen 1994).

Ingestion of forage containing condensed tannin (CT) by ruminants can improve their nitrogen use efficiency by reducing protein degradation in the rumen, reducing urine urea

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concentration and overall nitrogen excretion (Waghorn et al. 1987, 1994) and shifting more of the excreted nitrogen to the feces, where it is less likely to be lost to the air or groundwater. The optimal daily intake of CT when ingested as a constituent of forage appears to be 2–4% of dry-matter (DM) intake (Waghorn and Shelton 1995; Min et al. 2003). However, this conclusion may be altered somewhat depending on the type of CT consumed, if the amount of plant protein ingested is greater than that needed to maintain rumen microbial populations, and the accuracy of the analytical procedure used to measure CT. For grazing cattle and sheep, the benefits described above are limited by lack of CT in most grasses and important herbaceous forage species (e.g., alfalfa, *Medicago sativa* L.). Furthermore, there are significant challenges associated with establishing and/or maintaining pastures with herbaceous forages such as birdsfoot trefoil (*Lotus corniculatus* L.) and sainfoin (*Onobrychis viciaefolia* Scop.) that contain CT. For penned cattle and sheep, obtaining adequate intake of CT is possible by mixing it with their feed, but obtaining appropriate and uniform intake of a feed supplement containing CT may be difficult when cattle and sheep are grazing (Bowman and Sowell 1997). Therefore, the possibility of putting CT in drinking water to deliver small amounts of CT to all ruminants in a pasture is worthy of evaluation, especially considering that CT can be ingestion deterrents (Villalba and Provenza 2001). Potential outcomes include a reduction in urine urea excretion and improvements in nitrogen use efficiency.

Intake trials with sheep and cattle have demonstrated that ruminants can have normal intake of water when it contains low levels of CT (Kronberg 2008, 2010). The objectives of the research reported herein were to determine how much urine urea could be reduced for sheep and cattle when they ingested with water small amounts of a type of CT called quebracho tannin (QT) and if reductions in urine urea concentrations would occur relatively quickly after ingesting this type of CT in this manner. A quick response to CT ingestion is important because forage crude protein (CP) concentration may only be high enough to justify CT use for 1–2 $mo \cdot yr^{-1}$ when grazing arid and semiarid lands. Also, observations from an earlier study (Kronberg 2010) indicated that about 2% of DM intake of a crude QT extract (the same source of QT used in this study) may be the highest concentration that some ruminants can tolerate when ingested with water.

METHODS

Trial 1: Effect of CT Ingestion in Water on Blood Urea Concentration in Sheep

Urea concentrations in blood and urine are correlated (e.g., Zanton and Heinrichs 2009). To obtain an initial indication of the effect of CT ingestion, blood samples were collected during the trial described by Kronberg (2008) using four lambs and five QT concentrations. QT was obtained from the Tannin Corporation (Peabody, MA). It was about 85% CT and was used for all trials in this study. At the end of the pretrial period of tap water intake and on the morning of the eighth trial day for each concentration of QT offered, a jugular blood sample was collected from each lamb. The blood samples were refrigerated for about 2 h to clot before they were centrifuged ($1\,877 \times g$) and serum was collected from each sample. Serum samples from each lamb were stored frozen in sealed tubes until the end of the trial then analyzed for urea concentration (Sigma Diagnostics, Inc., St. Louis, MO). Data were analyzed using PROC Mixed (SAS 1996) with repeated measures of serum urea concentration and an autoregressive covariance structure used to determine if water type influenced urea concentration. Animal was the random variable in the model.

Trial 2: Effect of CT Ingestion in Water on Blood Urea Concentration in Cattle

Blood samples were collected during the trial described by Kronberg (2008) using 10 steers and seven QT concentrations. At the end of the 4-d tap water period and late on the morning of the final day of intake of the 1%, 1.5%, and 2.0% solutions of QT-water, a jugular blood sample was collected from each steer. The blood samples were refrigerated for about 2 h to clot before they were centrifuged ($1\,877 \times g$) and serum was collected from each sample. Serum samples from each steer were stored frozen in sealed tubes until the end of the trial then analyzed for urea concentration (Sigma Diagnostics). Data were analyzed as in trial 1.

Trial 3: Effect of CT Ingestion in Water on Excretion of Urine Urea and Fecal N in Sheep

To obtain a more detailed understanding of how QT ingestion in water would influence nitrogen excretion, four yearling castrated Rambouillet sheep (64.8 ± 5.4 kg) were kept in individual metabolism stalls in a barn maintained at 7.2°C. They were fed alfalfa pellets (21.9% CP, DM basis) at 2.5% of their body weight (BW) $\cdot\, d^{-1}$ with half their daily ration fed at 0630 hours and the other half fed at 1530 hours. Using a Latin-square experimental design, each sheep was offered four different liquids (one per period) to drink in ad libitum amounts from a self-activated drinking bowl, and daily intake of liquid was measured with a water meter placed on the supply line. The four liquids were tap water or tap water with concentrations of QT defined as low (0.5% of daily DM intake [DDMI], assuming that they drank similar amounts of this liquid as they did of tap water), medium (1.0% of DDMI with same assumption), or high (1.5% of DDMI with same assumption). The highest concentration of QT was reduced from 2.0% to 1.5% DDMI for this trial because in an evaluation of QT-water intakes (Kronberg 2010), some sheep appeared sick and reduced their feed intake while ingesting a QT-water solution of about 1.25% of DDMI. For each of four periods, an 8-d adaptation phase preceded a 2-d collection phase in which daily urine output was measured and sampled with 12-h collection intervals as was feces. Fifty milliliters of concentrated ($> 51\%$) sulfuric acid was placed in the urine collection containers to prevent loss of urea. Urine samples were promptly frozen after collection and analyzed for urea concentration (Sigma Diagnostics). Mean urine urea output ($g \cdot d^{-1}$) for both days of each period was determined, as was forage nitrogen intake ($g \cdot d^{-1}$), to produce the dependent variable daily urine urea excretion as a percentage of daily nitrogen intake. Daily fecal nitrogen excretion ($g \cdot d^{-1}$) was also determined for both days of each period. These data were analyzed as a 4×4 Latin square experiment

Table 1. Concentrations of urea in blood serum of castrated male lambs drinking either tap water or tap water with quebracho tannin (QT). Doses of QT were 0.25% (defined as very low QT), 0.5% (defined as low QT), 1.0% (defined as medium QT), and 2.0% (defined as high QT) of their daily alfalfa pellet intake as QT (assuming that they had similar intakes of these solutions as during the period of tap water intake, which they did).

	Serum urea ($mg \cdot dl^{-1}$)	
Type of water ingested	Mean[1]	SE
Tap water	19.0^a	0.41
Very low QT	19.0^a	0.41
Low QT	18.8^a	0.48
Medium QT	19.8^a	0.95
High QT	16.5^b	0.50

[1]Means with different letters are different ($P \leq 0.01$).

Table 2. Concentrations of urea in blood serum of castrated male cattle drinking either tap water or tap water with quebracho tannin (QT). Doses of QT were 1.0% (defined as medium QT), 1.5% (defined as medium plus QT) and 2.0% (defined as high QT) of their daily alfalfa pellet intake as QT (assuming that they had similar intakes of these solutions as during the period of tap water intake, which they did).

	Serum urea ($mg \cdot dl^{-1}$)	
Type of water ingested	Mean[1]	SE
Tap water	22.1^a	0.53
Medium QT	19.3^b	0.42
Medium plus QT	19.9^b	0.53
High QT	19.8^b	0.63

[1]Means with different letters are different ($P < 0.01$).

using PROC Mixed (SAS 1996) with animal as the random variable.

RESULTS AND DISCUSSION

Trial 1: Effect of CT Ingestion in Water on Blood Urea Concentration in Sheep

Mean serum urea concentration in the sheep was reduced when they ingested water with QT ($P = 0.03$) but only when they drank the mixture with the highest amount of QT (Table 1). Hervás et al. (2003) dosed sheep intraruminally with QT in water once daily for 21 d and observed that jugular blood plasma urea concentrations were lowered by the QT on the third and fifth days of dosing but not on the ninth day, and the reduced urea in plasma was seen only with the sheep given the highest level of QT, which seriously harmed them. This level was 3 $g \cdot kg^{-1}$ live weight, or about 156 g $QT \cdot d^{-1}$, which was much higher than our highest amount, which was about 40 g $QT \cdot d^{-1}$. When the sheep in the Hervás et al. (2003) study were dosed 26 or 78 g $QT \cdot d^{-1}$, no changes in plasma urea concentrations were observed, nor were indications of toxicosis observed. However, they did not report daily feed intake, so it is not possible to determine and compare QT levels as a percentage of feed or DM intake.

Trial 2: Effect of CT Ingestion in Water on Blood Urea Concentration in Cattle

Mean serum urea concentration was reduced in the steers when they drank water with QT in it ($P < 0.001$), but greater reduction (beyond 13%) in serum urea concentration was not observed with ingestion of QT concentrations greater than 1.0% of feed intake ($P \geq 0.27$; Table 2). Lactating dairy cows dosed twice daily with a total of 163 $g \cdot d^{-1}$ of CT extract (mixed 1:1 with water) from the black wattle tree (*Acacia mearnsii* de Wild) had 26% of feed nitrogen lost in urine compared to 39% lost when CT was not given (Grainger et al. 2009). However, when Grainger et al. (2009) dosed another group of cows with 244 $g \cdot d^{-1}$ of the CT extract, the reduction in feed nitrogen lost in urine was small (22% vs. 26% of feed nitrogen lost). The cattle in the present study ingested about 110, 165, and 220 $g \cdot d^{-1}$ of QT when drinking the three highest concentrations of QT-water offered them, so results from this trial and Grainger et al. (2009) indicate little benefit from increasing the amount of CT in drinking water of grazing cattle from 1% to 2% of their daily intake of DM. Also, Grainger et al. (2009) observed large reductions in dietary energy digestibility when either 163 or 244 $g \cdot d^{-1}$ of black wattle extract was dosed. Therefore, unless the CT from the black wattle tree is less than optimal CT to feed to ruminants, which is possible (Waghorn 2008), Grainger et al.'s results indicate that there are negative consequences for putting too much CT in water for cattle.

Trial 3: Effect of CT Ingestion in Water on Excretion of Urine Urea and Fecal N in Sheep

For the 2-d collection phase of the trial, mean liquid intakes (with SE) for the tap water, and low, medium, and high tannin solutions were 4.91 (2.12), 4.56 (1.77), 4.36 (1.53), and 4.48 (2.06) $L \cdot d^{-1}$, respectively. QT intake necessary to achieve the desired intake of tannin was 8.1, 16.2, and 24.3 $g \cdot d^{-1}$ for the low, medium, and highest QT treatments, respectively. The corresponding calculated intakes of tannin were 9.9, 19.4, and 29.2 $g \cdot d^{-1}$, or 0.6%, 1.2%, and 1.8% of daily DM intake, respectively. Therefore, intakes of all three QT solutions were more than adequate to obtain the desired intakes.

Drinking the low, medium, and high tannin solutions resulted in urine urea outputs (as a percentage of nitrogen intake) that were 3.5%, 6.6%, and 12.6% lower (linear; $P = 0.03$; Fig. 1), respectively, than urine urea output with tap water. Average daily fecal nitrogen excretion was 12.8, 14.0, 15.6, and 16.6 $g \cdot d^{-1}$ for the tap water and low, medium, and high tannin solutions, respectively ($P = 0.006$). There was also a linear effect of QT intake on fecal nitrogen excretion ($P = 0.001$). Although fecal nitrogen excretion was increased by QT ingestion, this nitrogen is not readily converted into volatile nitrogenous compounds as is urea in urine. Increased fecal nitrogen is more likely to contribute to the accumulation of nitrogenous material in the soil and be more valuable for integrated crop and livestock production.

These results are consistent with results from studies on sheep fed forages containing condensed tannins (Waghorn et al. 1987, 1994; Waghorn and Shelton 1995; Min et al. 2003). Our highest QT intake in trial 3 was within the beneficial range as suggested by Min et al. (2003), but our medium and low rates were below this range. Since our trial 3 data suggest that the medium and low rates of QT intake reduced protein

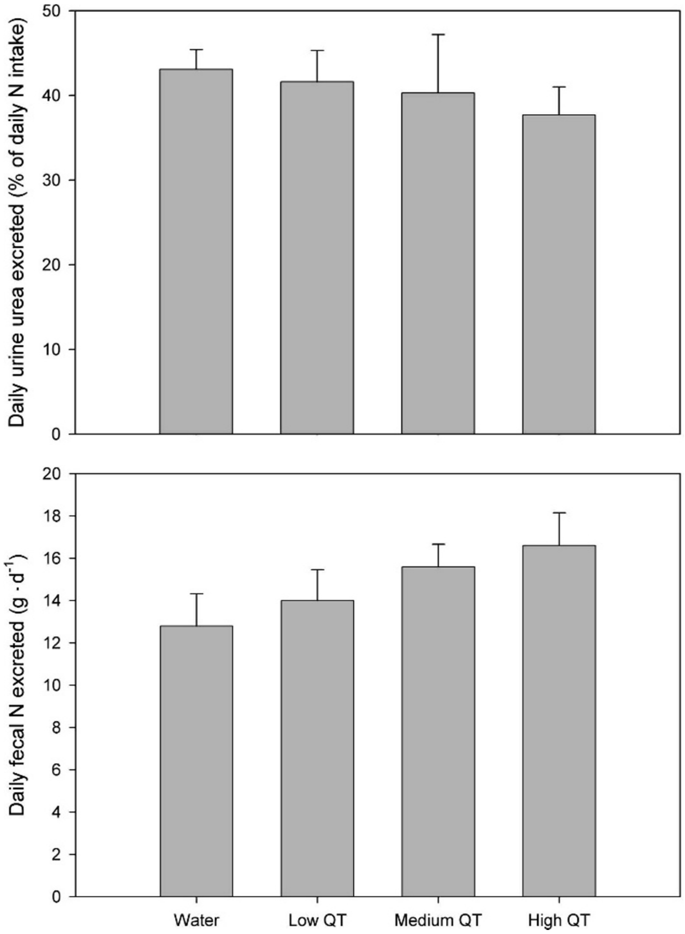

Figure 1. Daily urine urea excreted by sheep as a percentage of their daily nitrogen (N) intake and daily fecal N excreted by sheep when drinking tap water or tap water with low, medium, and high amounts of quebracho tannin (QT). The low, medium, and high concentrations of QT were designed to provide the sheep 0.5%, 1.0%, and 1.5%, respectively, of their daily dry-matter (DM) intake as QT. T-bars represent the standard error.

degradation in the rumen, ingestion of QT in water may increase its activity in the rumen compared to QT ingestion within forage, but delivering it via water may also result in QT having a negative impact on ruminant nutrition at lower concentration. Also, CT from many plants may reduce ammonia production in the rumen and consequently reduce urine urea production but not necessarily improve amino acid intestinal absorption and supply to the ruminant (Waghorn et al. 1987; Bermingham et al. 2001; Min et al. 2003; Waghorn 2008). On the basis of these studies, the preferred type of CT to put into stock water may be CT from birdsfoot trefoil or sulla (*Hedysarum coronarium* L.), which can reduce plant protein degradation in the rumen, increase protein flow to the small intestines, and increase amino acid absorption from the small intestines.

Our results indicate that ammonia and nitrous oxide emissions derived from urea in pasture urination spots may potentially be reduced by introducing small amounts of condensed tannin into the drinking water of ruminant livestock when these animals are consuming forage with high levels of CP. Although the short-term study of Liebig et al. (2008) did not observe lower cumulative nitrous oxide emissions from simulated urine spots from cattle drinking QT-water, it is possible that urination spots with lower nitrogen loads would emit less total nitrous oxide over the long term (Mosier et al. 1998). Additionally, ammonia emissions (as a percent of total nitrogen applied) were lower from soil when a mixture of feces and urine (in proportions excreted) was applied from cows consuming birdsfoot trefoil with high CT content (Misselbrook et al. 2005).

MANAGEMENT IMPLICATIONS

Results from this study indicate that small amounts of QT in the drinking water of grazing sheep can reduce their urine urea excretion onto pastures when they are consuming forage with high levels of CP, but further research is needed to determine if this reduction in urea excretion can lead to reduced amounts of ammonia and nitrous oxide emitted to the atmosphere. Also, research is needed to determine if other types of CT can be put into water to reduce urine urea excretion as well as improve animal productivity via changes such as improved amino acid absorption from the small intestine, reduced energy loss from methane eructation, and reduced internal parasite burden.

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