Sheep & Goat Research Journal
Volume 18: 2003

Contents:

Quebracho Tannin Influence on Nitrogen Balance in Small Ruminants and In-Vitro Parameters when Utilizing Alfalfa Forage

K.E. Turner* and J.P.S. Neel

Summary

Feeding studies using small ruminants and laboratory experiments were conducted to evaluate the level of quebracho tannin (QT) supplementation to alfalfa hay diets on plant protein nitrogen (N) use, organic matter (OM) and fiber disappearance, and *in vitro* ammonia production. In two separate feeding trials, sheep [Experiment 1; 12 crossbred wether lambs (avg wt 47.7 kg)] or goats [Experiment 2; 12 crossbred Boer wether kids (avg wt 32.7 kg)] were randomly assigned to one of four dietary treatments replicated three times. Lambs or kids were offered chopped alfalfa (*Medicago sativa* L.) hay supplemented with QT at 0.0, 0.75, 1.5, or 3.0% of the total dry matter (DM) intake. Sheep and goat diets containing 1.5 and 3.0% QT had higher ($P < 0.05$) fecal N excretion (g/d) than animals offered the 0.0 and 0.75% QT. As a result of greater fecal nitrogen loss, overall N digestibility was lower in sheep ($P < 0.07$) and goats ($P < 0.05$) offered the higher QT compared to 0.0 and 0.75% QT supplemented animals. Serum urea nitrogen was 11% lower in QT supplemented goats compared to goats offered no QT. Calculated in vivo neutral detergent fiber (NDF) digestibility was lower ($P < 0.10$) in lambs offered 1.5 and 3.0% QT diets when contrasted with lambs offered 0.0 and 0.75% QT. In laboratory studies using alfalfa hay incubated with QT, lag time for *in vitro* organic matter disappearance (IVOMD) was greatest ($P < 0.05$) for 3.0% QT when contrasted with 0.0, 0.75, and 1.5% QT, and at 48 h, IVOMD decreased quadratically($P < 0.05$) with QT addition. At 96 h, %NDF remaining decreased quadratically ($P < 0.07$) with QT additions. In vitro ammonia concentrations at 6 and 12 h were lower ($P < 0.05$) in tubes containing 1.5 and 3.0% QT when contrasted with tubes containing 0.0 and 0.75% QT. Further investigation is needed to define QT concentrations that allow optimal N and fiber utilization when ruminants are offered high protein, low energy diets or when grazing high quality pastures.

Key words: lamb, goat, quebracho tannin, nitrogen-use

Introduction

Forages supply N in both protein and non-protein nitrogen (NPN) forms for microbial protein synthesis. Proteolytic bacteria in the rumen metabolize excess NPN, protein, and amino acids (Nocek and Russell, 1988) to ammonia when there is limited carbohydrate (mainly water-soluble carbohydrate; Dove and Milne, 1994) availability or an asynchony of energy for incorporation into microbial protein (Beever, 1993; Sinclair et al., 1995). Excess rumen ammonia is eventually excreted via the urine as urea. Grazing ruminants can excrete 75-95% of ingested-N (Whitehead, 1970).

Secondary plant compounds (i.e. condensed tannins) found in specific plants can also improve N-use efficiency in ruminants (Mueller-Harvey, 1999). McSweeney et al. (1999) reported that proteolytic and peptidolytic ruminal bacteria from sheep and goats fed tannin-containing browse could not digest protein complexed with condensed tannins (CT). Low molecular weight CT bind dietary protein in the rumen thereby reducing excessive breakdown of protein by microorganisms and increasing the escape value of dietary protein (Barry, 1989). Since proteins are precipitated most effectively by CT at pH values near their isoelectric point (Hagerman and Klucher, 1986), the acidic conditions of the abomasum and upper small intestines should be conducive to digestion and absorption of the rumen-escape protein in the lower small intestines.

Forages containing 20-40 g CT kg^{-1} dry matter (DM) increased rumen escape protein value of herbage and improved livestock weight gain (Barry, 1985; Terrill et al., 1992). However, Barry (1989) reported that condensed tannin concentrations in plant tissues of greater than 4% restricted voluntary intake by lambs and depressed fiber digestion in the rumen.

Quebracho tannin is a commercial source containing a mixture of CT obtained from the red quebracho or quebracho colorado (*Schinopsis lorentzii*) tree and contains about 75% total tannins. Quebracho has been used as a feed additive to improve ruminant digestive utilization of protein rich soybean meal (Frutos et al., 2000). Information on the level of QT for small ruminants is lacking.

Our objective was to evaluate different levels of a commercially available source of condensed tannins (i.e. quebracho tannin) used as a supplement on N balance in lambs and kids offered high-protein hay diets, and to evaluate QT influences on OM disappearance, fiber breakdown, and ammonia production *in vitro*.

* USDA, ARS, Appalachian Farming Systems Research Center, 1224 Airport Road, Beaver, WV 25813. Corresponding author Ken.Turner@ars.usda.gov

Materials and Methods

Experiment 1 and 2: Metabolism Trials

In two separate digestion trials, sheep [12 crossbred wether lambs (avg wt 47.7 kg)] or goats [12 crossbred Boer wether kids (avg wt 32.7 kg)] were randomly assigned to one of four dietary treatments replicated three times. Lambs or kids were placed in stainless steel metabolism crates and housed in the Small Ruminant Housing Facility at USDA, ARS, AFSRC, Beaver, WV. Animals had access to water at all times. Protocols were approved by the Institutional Animal Care and Use Committee, USDA, ARS, AFSRC, Beaver, WV.

In both metabolism trials, chopped alfalfa (ALF) hay was offered as a base diet plus a supplement containing ground ALF hay, 10% dry molasses, and variable concentrations of quebracho vegetable extract (Tannin Corporation, Peabody, MA[1]). Supplements were offered at a constant percentage of DM intake to insure that total DM intake contained: 1) no supplemental quebracho tannin (0QT); 2) 0.75% quebracho tannin (0.75QT); 3) 1.5% quebracho tannin (1.5QT); or 4) 3.0% quebracho tannin (3.0QT). Nutritive value parameters of hays and supplements used in Experiment 1 and 2 are presented in Table 1.

Lambs and kids were adjusted to the metabolism crates for 2 d and then adapted to treatments diets for an additional 10 d followed by a 4-d total collection of feces and urine. Supplements with and without QT were offered first prior to feeding hay to insure intake of QT. During the collection period, animals were offered hay adjusted to 90% of the pervious 10-d voluntary intake to insure that QT was kept at a constant percentage of DM intake and to eliminate or minimize orts. Hay and supplement samples were collected throughout the course of the trial and composited separately. During the 4-d of intensive collections, total feces and urine were collected in plastic buckets. Total feces were weighed, a subsample obtained, weighed, and dried at 100°C, and then used to determine total fecal DM. A second fresh fecal sample (5% of total fresh weight) was dried at 60° C in a forced-air oven for about 5 d, and later composited by animal across days. Total urine volume measured prior to taking a 10% aliquot and composited for each animal across days. Urine samples were acidified with 0.5 N HCl and stored at -20° C. Blood samples were collected via jugular venipuncture at the beginning and end of the study, allowed to clot for 45 min, centrifuged to obtain serum, and stored at -20° C until analyzed.

Dried hay and supplement samples were ground to pass a 1-mm screen in a cyclone mill. Ground hay and supplements were analyzed for: DM and ash (AOAC, 1990); total N (Carlo-Erba Ea 1108 CHNS elemental analyzer[1], Fisons Instruments, Beverly, MA); NDF and ADF (Goering and Van Soest, 1970; Van Soest et al., 1991) using ANKOM[1] (Ankom Technology Corp., Fairport, NY) procedures; and IVOMD (Tilley and Terry, 1963; Moore, 1970; Goering and Van Soest, 1970). The IVOMD procedure used rumen fluid obtained from six ruminally cannulated does offered alfalfa and orchardgrass (*Dactylis glomerata* L.) hays. Total phenolic concentrations in the diets were determined as Folin-reactive phenolics using procedures of Hagerman and Butler (1986) in Experiment 1 only.

Dried fecal samples were analyzed for DM, total N, and NDF, and urine samples were analyzed for total N using techniques previously outlined above. Serum samples were analyzed for urea N (BUN) concentrations using automated procedures on the Ciba-Corning Express Plus Chemistry Analyzer[1] (Ciba-Corning Diagnostics Corp., Medfield, MA).

Experiment 3 and 4: In Vitro Laboratory Studies

Alfalfa (cv. Medistand) herbage was collected from a field experiment, composited, dried (60° C; forced-air oven), and ground to pass a 1-mm screen in a cyclone mill. Samples were analyzed for DM, ash, total N, NDF, ADF, and IVOMD using techniques as described above.

Experiment 3: In Vitro Organic Matter Disappearance of Herbage

Dried alfalfa herbage (0.5 g; DM basis) was weighed into 100-ml centrifuge tubes. Quebracho tannin solution with water [20% (w/v)] was added so that final concentrations were similar to those described for the sheep and goat metabolism studies: 1) 0 (0QT); 2) 0.75% (0.75QT); 3) 1.5% (1.5QT); or 4) 3.0% (3.0QT). Three replicates with duplicate tubes were used. Tubes containing samples, standards, and blanks were filled with 30 ml of a 2:1 mixture of McDougall's artificial saliva (McDougall, 1948) and goat rumen fluid (collected from ruminally cannulated does offered alfalfa and orchardgrass hay), and incubated at 39° C. The two-stage procedures of Tilley and Terry (1963) as modified by Moore (1970) and described by Turner et al. (1999) were used to determine rate of IVOMD (Goering and Van Soest, 1970) after 0, 6, 12, 24, 48, 72, and 96 h of total incubation time.

Experiment 4: Herbage NDF In Vitro Disappearance Rate and Ammonia Production

Dried alfalfa herbage (0.5g; DM base) was weighed into 100-ml centrifuge tubes and quebracho tannin solution [20% (w/v)] was added to create final QT concentrations as described above. Three replicates with duplicate tubes were used. Tubes were incubated at 39° C with McDougall's artificial saliva (McDougall, 1948) and rumen fluid using procedures similar to those of Cordes et al. (1988) using ruminal fluid from does used in Experiment 3. Tubes were incubated for 0, 6, 12, 24, 48, 72, and 96 h. When incubations were terminated, an ion specific electrode (Accumet, ammonia combination ion-selective electrode, Fisher Scientific, Pittsburgh, PA[1]) was used to measure ammonia concentrations in the tubes prior to determining NDF. The NDF content was measured using methods outlined by Turner et al. (1999). The IVOMD and NDF data were fitted to the natural log-linear regression model of Moore and Cherney (1986). Differences in parameter values were determined using ANOVA procedure of SAS (1990). Means were separated using Duncan's multiple range test.

Data in Experiment 1 and 2 were analyzed using GLM or ANOVA procedures of SAS

(1990). The model included effects for treatment and replicate (Snedecor and Cochran, 1980). In addition, data in Experiment 1 and 2 were evaluated for linear, quadratic, and cubic effects for changing QT concentrations. In Experiment 3 and 4, data were analyzed using the GLM procedures of SAS. The model included effects for treatment and replicate. Data were also evaluated for linear, quadratic, and cubic effects for NDF and IVOMD disappearance over time. Means in all experiments were separated when a significant ($P < 0.10$) *F*-value was indicated (Snedecor and Cochran, 1980).

Results

Experiment 1: Sheep Metabolism Trial

In Experiment 1, total N intake was similar (mean 20.2 g/d) among sheep (Table 2). Sheep offered the diets containing 1.5 and 3.0QT had higher ($P < 0.05$) amounts of daily fecal N (g/d) than sheep offered the 0 and 0.75 QT. Daily urinary N output (g/d) was 10% lower but not significant in lambs offered the QT diets compared to those receiving no QT. Nitrogen balance was similar among groups (mean 6.5 g/d), but lambs offered 3.0QT tended to have the lowest amount of retained N (6.0 g/d) compared to the other groups. Calculated N digestibility was higher ($P < 0.07$) for 0 and 0.75QT-diets compared to 1.5 and 3.0QT-diets as a result of more fecal N being excreted by animals offered the 1.5 and 3.0QT diets. The BUN concentrations were similar among groups (mean 21.5 mg/dl).

The NDF intake was higher ($P < 0.08$) by sheep offered the 0 and 0.75QT when contrasted with sheep offered 1.5 and 3.0QT diets (Table 2). Fecal output of NDF was similar. Calculated in vivo NDF digestibility was lower ($P < 0.10$) in lambs offered 1.5 and 3.0QT diets when contrasted with lambs offered 0 and 0.75QT.

Experiment 2: Goat Metabolism Trial

In Experiment 2, trends were similar to those in Experiment 1 (Table 2). Total N intake (mean 11.3 g/d) was similar ($P > 0.10$) among groups. The N intake for the 0.75QT group was numerically much lower compared to the other treatments, being a direct result of two animals refusing to consistently consume a large portion of the feed on offer. Total fecal N output (g/d) was higher ($P < 0.05$) for kids offered 1.5 and 3.0QT diets compared to goats offered the 0 and 0.75QT diets. Urinary N output was similar among groups (mean 4.0 g/d). Nitrogen balance was variable, but similar among groups (mean 0.57 g/d). Nitrogen digestibility was higher ($P < 0.05$) for 0 and 0.75QT compared to 1.5 and 3.0QT diets. The BUN were 11% lower, but not significant in kids offered QT containing diets compared to those offered no QT. The NDF intake, fecal NDF, and calculated NDF digestibility were not different ($P > 0.10$) among QT treatments.

Experiment 3: In Vitro Organic Matter Disappearance of Alfalfa Herbage

At 48 h, IVOMD decreased quadratically ($P < 0.05$) with QT addition (Fig. 1). Lag time was greatest ($P < 0.05$) for 3.0QT when contrasted with 0.0, 0.75, and 1.5QT (Table 3). Overall rate and extent of OM disappearance (Table 3) was not different ($P > 0.10$), but tended to be slower for 3.0QT (0.035) compared to 0.0, 0.75, and 1.5QT (mean 0.044).

Experiment 4: Alfalfa Herbage In Vitro NDF Disappearance and Ammonia Production

Linear ($P < 0.001$), quadratic ($P < 0.001$), and cubic ($P < 0.001$) effects for %NDF remaining at the specific h were evident with the least ($P < 0.001$) remaining after 96 h of incubation (data not shown). At h 96, %NDF remaining decreased quadratically ($P < 0.07$) with QT additions (Fig. 2). Rate of NDF disappearance was similar among treatments, but as with IVOMD, tended to be slowest for 3.0QT (Table 3). The 3.0QT treatment had the longest ($P < 0.05$) lag time when contrasted with 0, 0.75, and 1.5QT.

Ammonia concentrations measured at the specified h are presented in Fig. 3. There was a linear ($P < 0.001$) trend in ammonia production over time. Overall, ammonia concentrations for 1.5 and 3.0QT (mean 305 ppm) were lower ($P < 0.05$) compared to no QT (358 ppm); 0.75QT was intermediate (341 ppm). Time was a significant ($P < 0.01$) factor in ammonia production. As expected, ammonia concentrations were similar among the QT treatments ($P > 0.1$) at 0 h (mean 195 ppm). At 6 h, 1.5 and 3.0QT (mean 152 ppm) had lower ($P < 0.01$) ammonia concentrations than 0 and 0.75QT (mean 186 ppm) and ammonia concentrations decreased in a quadratic trend ($P < 0.01$) as QT concentration increased. A similar trend was observed at 12 hr, 1.5 and 3.0QT (mean 265 ppm) were lower ($P < 0.05$) compared with 0 and 0.75QT (mean 315) and decreased quadratically ($P < 0.01$) with increasing QT concentration. At 96 h, 1.5 and 3.0QT ammonia concentrations (mean 407 ppm) were lower ($P < 0.06$) than 0QT (504 ppm); 0.75QT was intermediate (456 ppm). Overall, ammonia production decreased quadratically ($P < 0.01$) as the concentrations of QT increased (Fig. 4).

Discussion

The lack of statistical differences among treatment groups for many of the parameters in our experiment was probably caused by inadequate replication; in the matabolism studies the three replicates that were used may not have accounted for high individual animal variation, especially on the goat metabolism trial. There was some variation in urine ouput and daily feed intakes even when intake was restricted to 90% of the previous 10-d by animals used in our two experiments. Dawson et al. (1999) reported that sheep offered grass hay diets containing 5% quebracho excreted an average of 18% more fecal DM and 41% less urine volume than control animals. Consequently, lambs offered tannin containing diets excreted more fecal N and less urinary N. Although not significant in our experiment, sheep offered QT excreted an average of 13% more feces and 40% more urine volume (data not shown) compared to no QT; a similar, not significant, trend was exhibited by the goats used in our experiment.

Dawson et al. (1999) showed *in vivo* NDF digestibility was reduced by about 7% after

2 weeks and 11% after 6 weeks of feeding quebracho to lambs. Our results revealed a negative trend in lambs and none in kids with regards to quebracho feeding and NDF digestion; this may be indicative that goats are better able to tolerate higher concentrations of tannins in their diet. It may also mean that it takes longer for negative effect from QT to build up in goats.

Increased fecal-N concentrations and decreased *in vivo* NDF digestibility associated with QT addition to the diet that we observed in our sheep experiment and *in vitro* study have been suggested to arise from 1) digesta protein bound to tannin, 2) decreased ruminal and intestinal digestive enzyme activity due to tannin, 3) impaired intestinal function, and 4) increased secretion of endogenous proteins (Butter et al., 1999).

Preston et al. (1965) and Pfander et al. (1975) reported high positive correlation between dietary N (protein) level and BUN. Blood urea-N concentrations averaged 19.0 mg/dl when dairy goats were offered an alfalfa meal-based diet (Gelaye and Amoah, 1991). Hatfield et al. (1998) reported higher BUN when lambs were offered a 18% CP diet compared to a 10% CP forage-based diet. Sahlu et al. (1993) reported that BUN increased as dietary CP level increased from 9 to 21% and meat type goats had the highest BUN (23.9 mg/dl) compared to milk or mohair type goats. The BUN concentrations from animals used in our study were not affected by dietary QT addition even though fecal N concentration increased with QT addition. Therefore, it would appear that excessive dietary N was not a factor on BUN in our animal feeding experiments.

Condensed tannins and other polyphenolics contained in quebracho probably had an initial anti-bacterial effect on microbes that slowed lag times (i.e., the time until active digestion by bacteria once in contact with substrate) and delayed the start of OM and NDF breakdown. This effect was enhanced as QT concentration was increased in the system. Condensed tannins and other compounds in QT may be inhibiting important bacterial cellulases and proteases. Salawu et al. (1999) reported reduced cellulase activity in rumen fluid from sheep offered 5% QT compared to no QT. We did not observe any differences in rate of *in vitro* OM and NDF disappearance, but amount of OM and NDF remaining at specific times was reduced, especially with 3.0QT compared to 0QT, suggesting that the 3.0QT did interfere with fiber breakdown. Sheep offered the higher levels of QT had lower *in vivo* NDF disappearance. We also observed reduced ammonia concentrations as QT was added to the system suggesting that ruminal protein breakdown was slowed or inhibited. Describing the ammonia production curve has been used as a measurement of proteolytic activity in the rumen (Orskov, 1992). In our experiment, ammonia concentrations decreased quadratically with QT addition. Hagerman et al. (1992) reported that digestible protein content decreased linearly when quebracho tannin was included in pelleted alfalfa diets offered to sheep. A linear decrease in digestible protein should result in a linear decrease in ammonia concentration since rate of ammonia production is directly related to the digestible protein fraction and rate of protein digestion. Broderick and Albrecht (1997) reported that differences in protein degradation rates of temperate forage legumes were proportional to condensed tannin concentrations. Condensed tannins can bind to proteins and peptides without altering protein structure (Broderick et al., 1991). The tannin-protein ratio (Hagerman and Robbins, 1987) becomes an important factor in regulating protein breakdown. In the sheep feeding study, the ratio of CP to total dietary phenolics ranged from 8.29 for alfalfa hay, and 7.87, 3.28, 2.55, and 1.31 for 0.0, 0.75, 1.5, and 3.0QT supplements, respectively. The alfalfa hay and hay-based supplements used in our animal feeding experiment were analyzed for total reactive phenolics, therefore we cannot differentiate between non-tannin and tannin effects on fiber digestibility. Energy concentrations were not evaluated in our study which may have influenced overall nutrient use. In Experiment 3 and 4, the alfalfa herbage used was of higher quality (higher total N and IVOMD, and lower NDF) compared to the alfalfa hay used in the animal feeding studies which probably resulted in different CP:QT ratios; total phenolics were not measured in the *in vitro* studies. The observed reduction in ammonia concentrations *in vitro* was probably related to QT's condensed tannin fraction effectively binding plant protein, thus protecting the protein from breakdown by microbes. The condensed tannin-protein complex may become too bulky to fit into the active site of proteolytic enzymes, thereby reducing protein degradability in the rumen, and increasing rumen-escape value of forage protein. Once in the abomasum, the environment favors dissociation of the condensed tannin-protein complex, but with increasing concentrations of QT (i.e. increasing condensed tannin concentrations) in the diet, a higher proportion of plant protein, microbial protein, and bacterial and mammalian digestive enzymes remain bound to the condensed tannin fraction, resulting in higher total fecal nitrogen, decreased nitrogen digestibility, and decreased NDF digestibility, as was observed in our animal feeding experiments.

Conclusions

In our experiment, 1.5 and 3.0QT supplementation to alfalfa forage resulted in decreased NDF digestibility in sheep, and more total fecal N being excreted by sheep and goats compared to no QT supplementation. We also observed decreased IVOMD and NDF disappearance and lower ammonia concentrations when higher concentrations of 1.5 and 3.0QT were added to alfalfa substrate and incubated with rumen fluid *in vitro* compared to no QT. Further investigation is needed to define QT concentrations that allow optimal N and fiber utilization when ruminants are offered high protein, low energy diets, or when grazing high quality pastures.

Acknowledgements

We thank J.P. Snuffer, J.B. Ellison, K.N. Harless, and R. Lester for technical assistance in animal care, data collection, and laboratory analyses.

1 Trade or product names are mentioned for the convenience of the reader and do not imply endorsement over comparable products or equipment.

Literature Cited

AOAC, 1990. Official methods of analysis. 15th ed. AOAC, Washington, DC.

Barry, T.N. 1985. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 3. Rates of body and wool growth. Br. J. Nutr. 54:211-217.

Barry, T.N. 1989. Condensed tannins: Their role in ruminant nutrition and carbohydrate digestion and possible effects upon rumen ecosystem. pp. 153-169. In: J.V. Nolan, R.A. Leng, and I.D. Demeyer (eds.) The Roles of Protozoa and Fungi in Ruminant Digestion. Penambul Books, Armidale, NSW, Australia.

Beever, D.E. 1993. Rumen function. In: J.M. Forbes and J. France (eds). Quantitative Aspects of Ruminant Digestion and Metabolism. CAB International, Wallingford, Oxon, UK.

Broderick, G.A., and K.A. Albrecht. 1997. Ruminal in vitro degradation of protein in tannin-free and tannin-containing forage legume species. Crop Sci. 37:1884-1891.

Broderick, G.A., R.J. Wallace, and E.R. Orskov. 1991. Control of rate and extent of protein degradation. Pp. 541-592. In: T. Tsuda, Y. Sasaki, and R. Kawashima (eds.) Physiological Aspects of Digestion and Metabolism in Ruminants. Academic Press, Orlando, FL.

Butter, N.L., J.M. Dawson, and P.J. Buttery. 1999. Effects of dietary tannins on ruminants. Pp. 51-70. In: J.C. Caygill and I. Mueller-Harvey (eds.) Secondary Plant Products: Antinutritional and beneficial actions in animal feeding. Nottingham Univ. Press, United Kingdom.

Cordes, C.S., K.E. Turner, J.A. Paterson, J.G.P. Bowman, and J.R. Forwood. 1988. Corn gluten feed supplementation of grass hay diets for beef cows and yearling heifers. J. Anim. Sci. 66:522-531.

Dove, H., and J.A. Milne. 1994. Digesta flow and rumen microbial protein production in ewes grazing perennial ryegrass. Aust. J. Agric. Res. 45:1229-1245.

Dawson, J.M., P.J. Buttery, D. Jenkins, C.D. Wood, and M. Gill. 1999. Effects of dietary quebracho tannin on nutrient utilisation and tissue metabolism in sheep and rats. J. Sci. Food Agric. 79:1423-1430.

Frutos, P., G. Hervas, F.J. Giraldez, M. Fernandez, and A.R. Mantecon. 2000. Digestive utilization of quebracho-treated soya bean meals in sheep. J. Agric. Sci. 134:101-108.

Gelaye, S., and E.A. Amoah. 1991. Nutritive value of florigraze rhizoma peanut as an alternative leguminous forage for goats. Small Ruminant Res. 6:131-139.

Goering, H.K, and P.J. Van Soest, 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). USDA-ARS Agric. Handb. 379. Washington, DC.

Hagerman, A.E., and L.G. Butler. 1986. Assay of condensed tannins or flavonoid oligomers and related flavonoids in plants. Methods Enzymol. 234:429-437.

Hagerman, A.E., and K.M. Klucher. 1986. Tannin-protein interactions. pp. 67-76. In: V. Cody, E. Middleton, and J. Harborne (eds.) Plant Flavenoids in Biology and Medicine: Biochemical, Pharmacological, and Structure Activity Relationships. Alan R. Liss, Inc., New York.

Hagerman, A.E., and C.T. Robbins. 1987. Implications of soluble tannin-protein complexes for tannin analysis and plant defense mechanisms. J. Chem. Ecol. 13:1243-1259.

Hagerman, A.E., C.T. Robbins, Y. Weerasuriya, T.C. Wilson, and C. McArthur. 1992. Tannin chemistry in relation to digestion. J. Range Manage. 45:57-62.

Hatfield, P.G., J.A. Hopkins, W.S. Ramsey, and A. Gilmore. 1998. Effects of level of protein and type of molasses on digesta kinetics and blood metabolites in sheep. Small Ruminant Res. 28:161-170.

McDougall, E.I. 1948. Studies on ruminant saliva: I. The composition and output of sheep's saliva. Biochem. 43:99-104.

McSweeney, C.S., B. Palmer, R. Bunch, and D.O. Krause. 1999. Isolation and characterization of proteolytic ruminal bacteria from sheep and goats fed the tannin-containing shrub legume *Calliandra calothyrsus*. Appl. Environ. Microbiol. 65:3075-3083.

Moore, J.E. 1970. Procedures for the two-stage in vitro digestion of forages. pp. 5001-5003. In: L.E. Harris (ed.) Nutrition Research Techniques for Domestic and Wild Animals. Vol. 1. Utah State Univ., Logan.

Moore, K.J., and J.H. Cherney. 1986. Digestion kinetics of sequentially extracted cell wall components of forages. Crop Sci. 26:1230-1235.

Mueller-Harvey, I. 1999. Tannins: Their nature and biological significance. pp. 17-39. In: J.C. Caygill and I. Mueller-Harvey (eds.) Secondary Plant Products: Antinutritional and Beneficial Actions in Animal Feeding. Nottingham Univ. Press, United Kingdom.

Nocek, J.E., and J.B. Russell. 1988. Protein and energy as an integrated system, relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71:2070-2107.

Orskov, E.R. 1992. Protein nutrition in ruminants. 2nd ed. Academic Press, Inc., San Diego, CA.

Pfander, W.H., S.E. Grebing, C.M. Price, O. Lewis, J.M. Asplund, and C.V. Ross. 1975. Use of plasma urea nitrogen to vary protein allowances of lambs. J. Anim. Sci. 41:647-653.

Preston, R.L., D.D. Schnakenberg, and W.H. Pfander. 1965. Protein utilization in ruminants. I. Blood urea nitrogen as affected by protein intake. J. Nutr. 86:281-288.

SAS. 1990. SAS User's Guide: Statistics. Version 6 ed. SAS Institute., Cary, NC.

Sahlu, T., S.P. Hart, and J.M. Fernandez. 1993. Nitrogen metabolism and blood metabolites in three goat breeds fed increasing amounts of protein. Small Ruminant Res. 10:281-292.

Salawu, M.B., T. Acamovic, C.S. Stewart, and F.D. DeB. Hovell. 1999. Effects of feeding quebracho tannin diets, with and without a dietary modifier, on rumen function in sheep. Anim. Sci. 69:265-274.

Sinclair, L.A., P.C. Garnsworthy, J. Newbold, and P.J. Buttery. 1995. Effects of synchronising the rate of dietary energy and nitrogen release in diets with similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. 124:463-472.

Snedecor, G.W., and W.G. Cochran. 1980. Statistical methods. 7th ed. Iowa State Univ. Press, Ames.

Terrill, T.H., G.B. Douglas, A.G. Foote, R.W. Purchas, G.F. Wilson, and T.N. Barry. 1992. Effect of condensed tannin upon body growth, wool growth, and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. J. Agric. Sci. (Cambr.) 58:312-329.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grasssl. Soc. 18:104-109.

Turner, K.E., D.P. Belesky, and J.M. Fedders. 1999. Chicory effects on lamb weight gain and rate of in vitro organic matter and fiber disappearance. Agron. J. 91:445-450.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Whitehead, D.C. 1970. The role of nitrogen in grassland productivity. Commonwealth Bureau Pastures and Field Crops Bull. 48. P. 202. Commonwealth Agric. Bureau, Farnham Royal.

Table 1. Composition alfalfa (ALF) hay and supplements with quebracho tannin (QT) offered to lambs (Experiment 1) and kids (Experiment 2) at 0, 0.75, 1.5, or 3 % of the total dry matter intake, and composition of ALF herbage used in the *in vitro* study (Experiment 3).

Component[a]	ALF Hay	0 QT Supplement	0.75QT Supplement	1.5QT Supplement	3.0QT Supplement
Experiment 1					
Total N, %	2.74	2.80	2.24	2.48	2.22
CP, %	17.1	17.5	14.0	15.5	13.9
NDF, %	48.2	49.6	50.6	42.9	49.4
ADF, %	41.9	37.2	38.0	43.9	35.1
IVOMD, %	52.7	59.6	49.4	53.8	54.3
Experiment 2					
Total N, %	2.57	2.40	2.31	2.35	2.26
CP, %	16.1	15.0	14.4	14.7	14.1
NDF, %	56.8	53.5	52.5	45.8	32.6
ADF, %	42.0	41.1	37.8	33.5	25.8
IVOMD, %	50.9	49.0	51.8	50.4	54.8
Experiment 3 and 4	ALF Herbage				
Total N, %	4.18				
CP, %	26.1				
NDF, %	28.1				
ADF, %	19.9				
IVOMD, %	69.3				

[a]Dry matter basis. N = nitrogen; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; IVOMD = in vitro organic matter disappearance.

Table 2. Nitrogen balance, blood urea nitrogen, and neutral detergent fiber (NDF) digestibility in sheep and goats offered alfalfa hay diets containing quebracho tannin (QT) at 0.0, 0.75, 1.5, or 3.0% of total dry matter intake.

		----------QT%----------				
Experiment						
1 (Sheep)	Item	0	0.75	1.5	3.0	Significant Contrast
	Total N Intake, g/d	20.3	20.4	20.4	19.7	
	Fecal N, g/d	6.0	6.5	7.2	6.8	a
	Urine N, g/d	7.7	7.4	6.3	7.0	
	N Balance, g/d	6.7	6.5	6.9	6.0	
	N Digestibility, %	70.6	68.2	64.8	65.5	b
	BUN, mg/dl	20.7	22.0	21.4	22.0	
	Total NDF Intake, g/d	369.5	389.6	353.3	355.1	c
	Fecal NDF, g/d	214.2	248.9	238.3	232.7	
	NDF Digestibility, %	42.1	36.1	36.6	34.7	d
2 (Goats)						
	Total N Intake, g/d	11.2	7.8	13.9	12.3	
	Fecal N, g/d	5.52	4.63	9.09	7.60	e
	Urine N, g/d	4.9	2.9	4.3	3.9	
	N Balance, g/d	0.76	0.21	0.46	0.86	
	N Digestibility, %	50.6	40.3	34.5	38.4	f
	BUN, mg/dl	25.3	22.2	22.2	23.3	
	Total NDF Intake, g/d	255.5	197.9	318.4	280.7	
	Fecal NDF, g/d	146.3	113.0	181.5	163.5	
	NDF Digestibility, %	41.6	44.0	43.0	41.7	

[a,e,f]Means in the same row, contrast 0 & 0.75 vs. 1.5 & 3.0, $P < 0.05$.
[b] Means in the same row, contrast 0 & 0.75 vs. 1.5 & 3.0, $P < 0.07$.
[c] Means in the same row, contrast 0 & 0.75 vs. 1.5 & 3.0, $P < 0.08$.
[d]Means in the same row, contrast 0 & 0.75 vs. 1.5 and 3.0; $P < 0.10$.

Table 3. Mean model parameters of organic matter disappearance (IVOMD) in Experiment 3 and in vitro neutral detergent fiber (NDF) disappearance in Experiment 4 when dried alfalfa (ALF) herbage was incubated with quebracho tannin (QT) at 0, 0.75, 1.5, or 3 % of the total dry matter.

Experiment	Item	QT % Treatment	Rate Constant	Lag	R^2	Extent of disappearance†
			h^{-1}	h		
3	IVOMD	0.0	0.0429	-1.9[a]	0.81	0.61
		0.75	0.0495	-4.4	0.90	0.63
		1.5	0.0455	-9.8	0.89	0.72
		3.0	0.0355	-5.6	0.89	0.64
4	NDF	0.0	0.0377	9.4[b]	0.93	0.64
		0.75	0.0327	9.3	0.90	0.65
		1.5	0.0308	8.8	0.86	0.65
		3.0	0.0292	10.6	0.86	0.66

†In Experiment 1, calculated as the ratio of OM remaining at t = 72 hr to OM at t = 0 h. In Experiment 2, calculated as the ratio of NDF remaining at t = 96 h to NDF at t = 0 h.
[a]Within an Experiment and within a column, contrast 0 & 0.75 & 1.5 vs. 3.0 QT ($P < 0.05$). Quadratic trend ($P < .05$).
[b]Within an Experiment and within a column, contrast 0 & 0.75 & 1.5 vs. 3.0 QT ($P < 0.05$). Quadratic trend ($P < 0.05$).

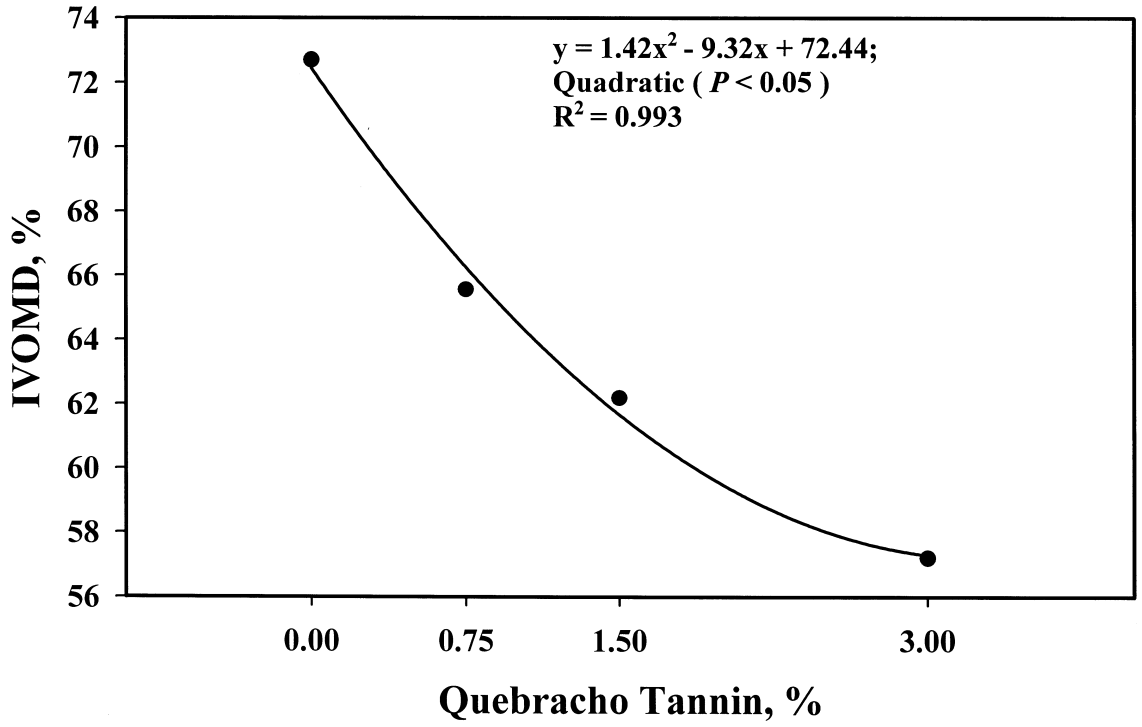

Figure 1. In vitro organic matter disappearance (IVOMD) at 48 h when alfalfa hay was incubated with 0.0, 0.75, 1.5, or 3% quebracho tannin (QT) in Experiment 3.

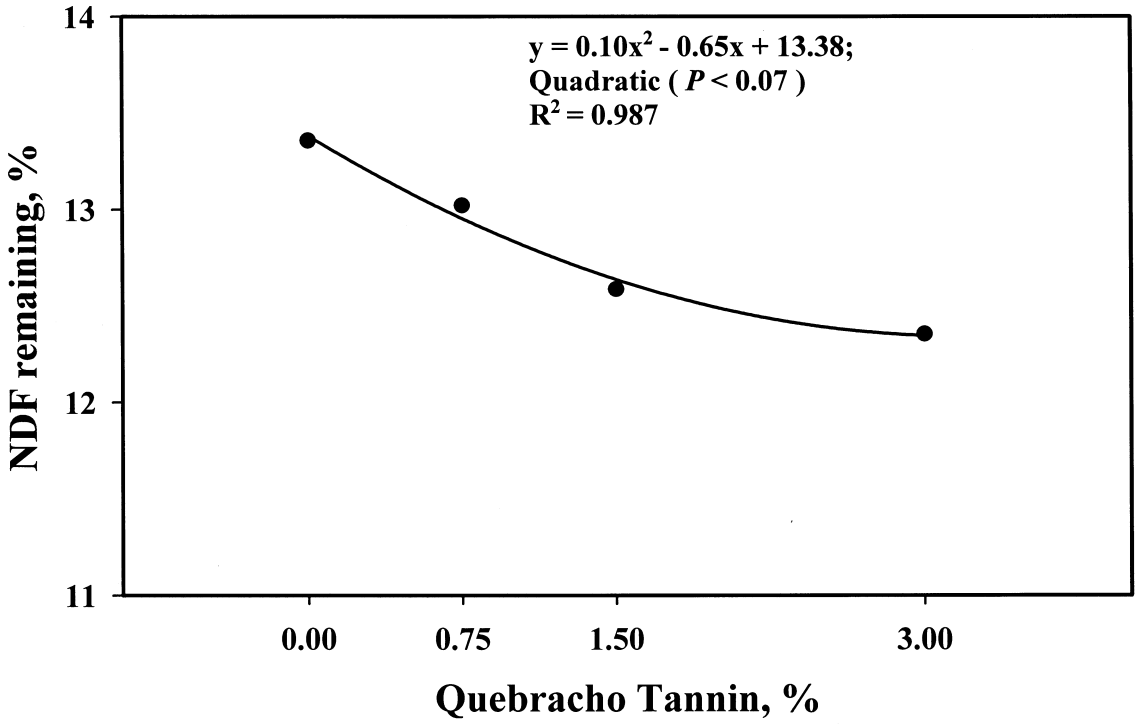

Figure 2. Percent neutral detergent fiber (NDF) remaining at 96 h when alfalfa hay was incubated with 0.0, 0.75, 1.5, or 3% quebracho tannin (QT) in Exp 4.

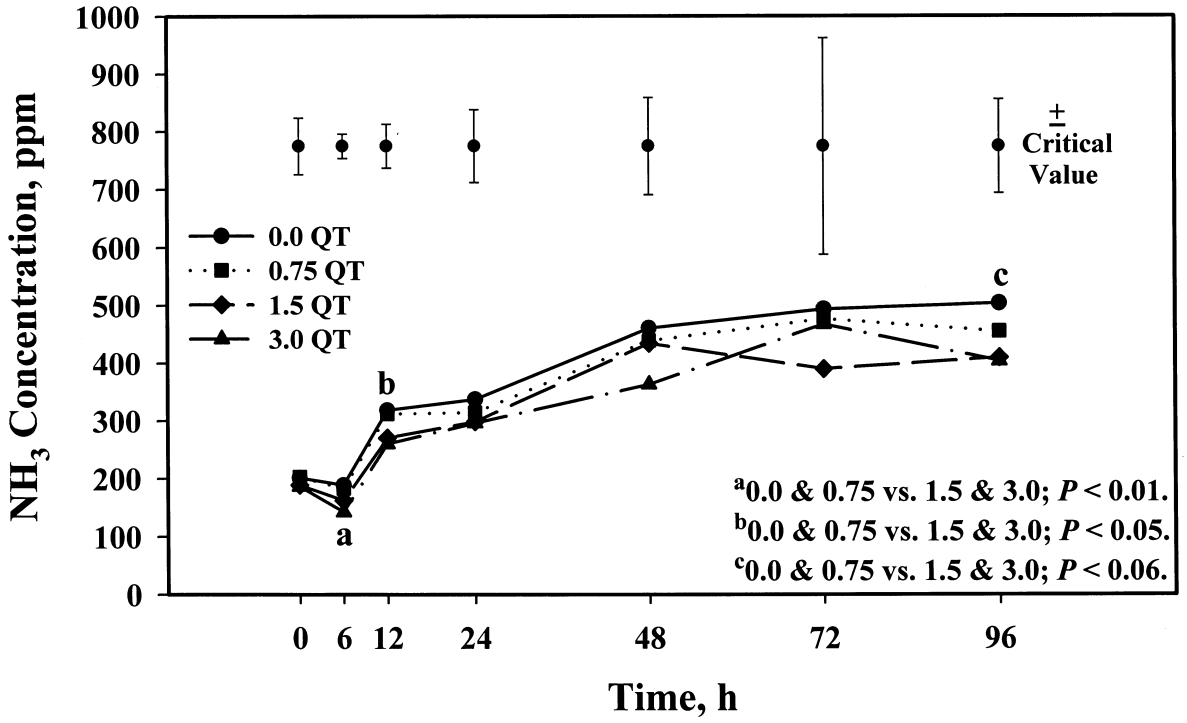

Figure 3. Ammonia (NH_3) concentrations over time when alfalfa hay was incubated with 0.0, 0.75, 1.5, or 3.0% quebracho tannin (QT) in Experiment 4. Critical values for treatment mean comparisons are shown. Significant contrasts of 0.0 & 0.75 vs 1.5 & 3.0 are presented.

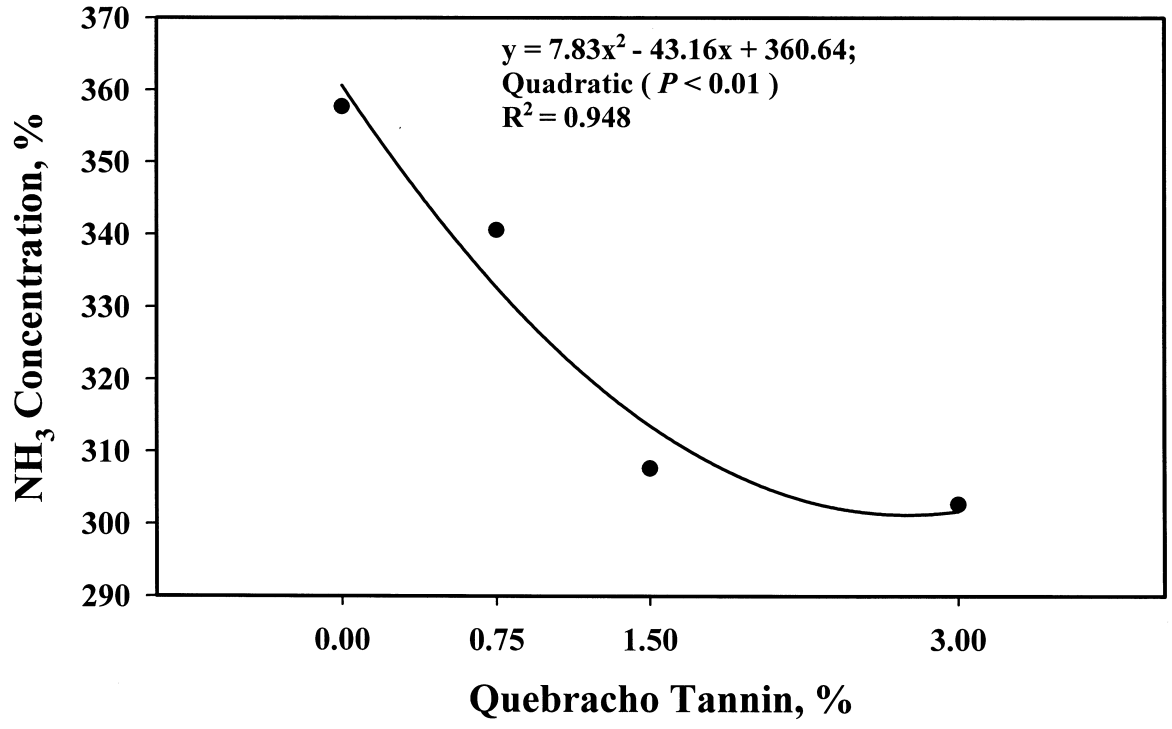

Figure 4. Overall pattern (quadratic; P < 0.01) of ammonia (NH_3) concentrations when alfalfa hay was incubated with 0.0, 0.75, 1.5, or 3% quebracho tannin (QT) in Experiment 4.