Short- to medium-term effects of consumption of quebracho tannins on saliva production and composition in sheep and goats

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Short- to medium-term effects of consumption of quebracho tannins on saliva production and composition in sheep and goats[1]

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ABSTRACT: Eight Merino sheep (49.4 ± 4.23 kg BW) and 8 Alpine goats (53.2 ± 2.51 kg BW) were used to study the effect of ingestion of quebracho tannins on salivation. Four sheep and 4 goats were individually fed a daily allotment of 20 g DM of alfalfa hay/kg BW (Control). Another 4 sheep and 4 goats were also given 20 g DM of alfalfa hay/kg BW supplemented with 50 g of quebracho/kg DM (Tannin) for a period of 64 d. The saliva secretion from the left parotid gland was collected by insertion of a polyvinyl chloride catheter into the parotid duct and the amount of parotid saliva produced recorded over three 48-h periods on d 1 and 2 (P1), d 31 and 32 (P2), and d 61 and 62 (P3) after the tannin feeding was initiated. The total amount of saliva produced was estimated from rumen water kinetics determined on d 4, d 34, and d 64 of the experiment. Experimental design was completely randomized, with repeated measures on each experimental unit, performing separate analysis for sheep and goats. Parotid saliva production was not affected by the sampling period in either animal species receiving the Control diet. Corresponding values for sheep were 2.04, 2.12, and 2.27 L/d ($P = 0.89$) and for goats 1.65, 1.79, and 1.86 L/d ($P = 0.95$). Sheep fed the Tannin diet produced 55, 73, and 107% of the amount of saliva recorded in sheep fed the Control diet on P1, P2, or P3, respectively. Corresponding values in goats were 88, 130, and 134% on P1, P2, or P3, respectively. Estimated total saliva production was not affected ($P = 0.50$ for sheep and $P = 0.97$ for goats) by the ingestion of quebracho. There was no difference ($P > 0.10$) in osmotic pressure, P, Mg, Ca, urea, and protein concentrations in parotid saliva. There were, however, differences in Na and K concentrations in response to the ingestion of quebracho tannins, with Na concentrations increasing ($P = 0.05$) and K concentrations decreasing ($P = 0.04$) in sheep saliva and pH increasing ($P = 0.05$) in goat saliva. In conclusion, the inclusion of quebracho at 50 g/kg DM for 64 d does not appear to alter saliva production in sheep and goats.

Key words: goats, quebracho tannins, saliva, sheep

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INTRODUCTION

Tannins are a complex group of secondary metabolites, which occur in different plant species commonly grazed by sheep and goats that can cause either beneficial or detrimental nutritional effects in animals (Mueller-Harvey, 2006). Herbivores have developed different mechanisms to counteract the negative effects of tannins (Estell, 2010) and saliva has been considered a first line defense against tannins ingested although the studies are not conclusive, especially in relation with sheep and goats (Lamy et al., 2011).

Considering the feeding types proposed by Hofmann (1989), browsers have a better ability to deal with tannins than grazers. Browsers have larger salivary glands than grazers, suggesting a potential to yield larger amounts of saliva. However, these size

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differences are not always related to saliva production (Robbins et al., 1995; Hofmann et al., 2008).

Sheep and goats are both generalist herbivores, but goats have been classified as being closer to browsers, with some potential ability to cope with the adverse effects of secondary compounds (Marsh et al., 2006), whereas sheep are classified as grazers. Vaithiyanathan et al. (2001) reported that goats have a greater parotid gland weight relative to BW and produce more parotid saliva (mL/g DMI; Seth et al., 1976) than sheep. These differences in parotid saliva production were not observed by González et al. (1999). Seth et al. (1976) reported different chemical composition in sheep and goat parotid saliva, but Lamy et al. (2008) observed a strong similarity between the electrophoretic profiles of sheep and goat salivary proteins.

We hypothesized that the presence or absence of tannins in the diet can determine salivation. Little information and no conclusive results have been reported on this topic. Therefore, the purpose of this study was to investigate the acute and medium-term impact of tannins on salivation (parotid saliva secretion and composition and total saliva production) in sheep and goats.

MATERIALS AND METHODS

The experimental procedures used were approved by the Institutional Animal Care and Use Committee at University of Leon (Spain) in compliance with the European and Spanish laws for the use of animals in research.

Animals and Experimental Design

Eight Merino sheep (49.4 ± 4.23 kg BW) and 8 Alpine goats (53.2 ± 2.51 kg BW) were individually housed in pens (1 by 1.10 m) in a continuously illuminated stall. All the animals were mature nonpregnant and nonlactating females and were fitted with a permanent rumen cannula 3 mo before the beginning of the experiments.

Animals were fed a maintenance diet (2% of BW as-fed) of chopped (3 to 4 cm length) alfalfa hay [with 134 g of CP, 538 g of NDF, and 354 g of ADF per kg of DM and estimated energy content (NRC, 2007) of 1.8 Mcal ME/kg DM] once daily at 0900 h. Orts for each animal were removed 10 min before the morning feeding and recorded daily. Animals had ad libitum access to fresh water and a mineral block (Tegablock; Inatega SL, Leon, Spain; 32% Na, 4% Ca, 0.1% Mg, 0.1% P, 0.1% Zn, 0.05% Mn, 0.002% I, 0.002% Se, and 0.001% Co). Animals were on a maintenance diet for a period of 15 d. At the conclusion of the 15-d adaptation period (d 0) animals were randomly assigned to 1 of 2 treatments: Control treatment in which the animals were fed the maintenance diet, which was sprayed with 200 mL distilled water/kg DM, and Tannin treatment in which animals were fed the maintenance diet, which was sprayed with 200 mL/kg DM of a distilled water solution containing 250 g quebracho (Roy Wilson Dickson Ltd., Alrewas, Stafordshire, UK; 760 g condensed tannins/kg) per liter of distilled water, thus providing 50 g quebracho/kg alfalfa DM, a dose that is under the level that causes health problems (Hervas et al., 2003) and that has been used in other studies (Dawson et al., 1999). Quebracho condensed tannins are flavonoid polymers extracted from *Schinopsis* spp. The solutions were sprayed onto the alfalfa hay corresponding to each animal and mixed thoroughly by hand just before feeding. The experiment lasted 64 d, starting the first day quebracho was administered to the animals of the Tannin group.

Measurements

Measurements were taken over 3 periods at the beginning [d 1 to d 4 (**P1**)], middle [d 31 to d 34 (**P2**)], and end [d 61 to d 64 (**P3**)] of the experiment. Within each period, collection of saliva was performed during the first 2 d (d 1 and 2, d 31 and 32, and d 61 and 62) and rumen samples the fourth day (d 4, d 34, and d 64).

The saliva secretion of the left (unilateral model) parotid gland was recorded for all animals. At the beginning of each period (d 0, d 30, and d 60), animals were anesthetized (injecting xylacine intramuscularly and ketamine intravenously), and sterilized polyvinyl chloride catheters (2.0 mm i.d. and 3.0 mm o.d.) were inserted into the left parotid duct (via its papilla in the mouth), brought to the surface of cheek, wrapped with adhesive tape, and secured to the skin with cyanoacrylate adhesive (Carter and Grovum, 1988; González and Grovum, 1993). Saliva was collected into a covered plastic container for 2 consecutive d (d 1 and 2, d 31 and 32, and d 61 and 62) and then weighed to determine daily saliva output. After determination of daily saliva output, pH was determined using a handheld pH meter (WTW pH91, Wissenschaftlich-Tecknische Werkstätten GmbH, Weilheim, Germany) and then saliva was immediately frozen at –20°C until further analysis for chemical composition and osmotic pressure.

Rumen fluid volume and outflow rate were estimated on d 4, d 34, and d 64 of the experiment by dosing 2 g of Co-EDTA (Udén et al., 1980) dissolved in 50 mL of distilled water via the cannula into several sites in the rumen. Thereafter, samples of rumen fluid (50 mL) were taken at 0, 1, 3, 5, 7, 9, 12, and 24 h from multiple sites of the rumen using a probe and a manual suction pump. Samples were strained through 4 layers of cheesecloth. An aliquot (0.8 mL) of strained rumen fluid was reserved for osmotic pressure

(**OP**) determination and the remaining sample for Co determination. Both samples were immediately frozen and stored at –20°C until corresponding analysis. Drinking water intake was recorded at all periods by measuring the amount of water added daily to restore the initial level of water in the drinker.

Chemical Analyses

Osmotic pressure was measured in saliva and rumen liquid samples by using a vapor pressure osmometer Wescor 5100c (Wescor Inc., Logan, UT). Total proteins (**TP**) and urea concentrations were determined using test kits in a Hitachi 704 auto-analyzer (Hitachi Ltd., Tokyo, Japan). One sample (0.5 mL) of parotid saliva was diluted (1:10) with 4.5 mL of distilled water and used to determine P, Mg, Ca, Na, and K concentrations. The determinations were performed using an Inductively Coupled Plasma-Atomic Emission Spectroscopy analyzer (Optima 200DV; PerkinElmer, Überlingen, Germany). The concentration of Co in centrifuged rumen fluid (2,500 × *g* for 20 min at 4°C) was determined by atomic absorption spectrophotometry (PerkinElmer 3110, Überlingen, Germany) at a wavelength of 240 nm.

Calculations and Statistical Analyses

Fractional liquid dilution rate ($\boldsymbol{k}_{\mathbf{liquid}}$; h^{-1}) was estimated as the slope of the linear regression between the natural log of Co concentration in the rumen fluid and the time of sampling after marker administration. Daily liquid outflow (***O***; L/d) was calculated as $O = k_{\text{liquid}} \times 24 \times V$, in which ***V*** is the rumen liquid volume (L) estimated from the intercept of the Co concentration curve.

Total daily saliva production (**TDSP**) was estimated as the difference between water outputs from the reticulorumen [absorption through the wall (***A***) and liquid outflow (*O*)] and measured inputs [drinking (***D***) and feed (***F***) water] taking into account the amounts of liquid added with the marker and withdrawn with samples (*c*):

$$\text{TDSP} = A + O - D - F \pm c.$$

The water absorption through the rumen wall was calculated from the OP using the equation $A = 395 - 1.16$ OP, proposed by López et al. (1994), considering the ruminal OP values recorded at all sampling times as described by Ranilla et al. (1998).

Experimental design was completely randomized, with animals considered as the experimental units. Data were analyzed separately for sheep and goats. The PROC MIXED procedure (SAS Inst. Inc., Cary, NC) was used for repeated measures analysis (Littell et al., 1998). The model used was

$$y_{ijk} = \mu + D_i + \delta_{j(i)} + P_k + DP_{ik} + \varepsilon_{ijk},$$

in which y_{ijk} is the response at period k on animal j in diet group i, μ is the overall mean, D_i is the fixed effect of diet i (i = Control or Tannin), $\delta_{j(i)}$ is the random effect of animal j within diet i (which was the term used as experimental error to test the effect of diet i), P_k is the effect of period k (k = P1, P2, or P3), DP_{ik} is the interaction effect of diet i with period k, and ε_{ijk} is the residual error (random error at period k on animal j in diet i).

The mixed model contains the between-animal random effect $\delta_{j(i)}$, and ε_{ijk} contains the within-animal residual error. The structure of the variance–covariance error matrix used was unstructured, based on Bayesian criteria observed with several alternative structures. Results reported in the tables and in the text are least-square means of fixed effects with their corresponding SE. Test of simple effects were used to partition (slice) interaction effects by diet to test effects of period separately for each diet. The data related to the protein content of the parotid saliva were log-transformed to obtain a normal distribution and homogenous residual error (Chiquette, 2009). Statistical significance was declared at $P \leq 0.05$.

RESULTS

Saliva produced by the left parotid gland of sheep (Table 1) was not affected by the inclusion of quebracho ($P = 0.31$) in the diet or by period ($P = 0.14$). There was no significant ($P = 0.30$) treatment × period interaction on parotid saliva production. Mean saliva outputs for Control sheep on P1, P2, and P3 were 2.04, 2.12, and 2.27 L/d ($P = 0.89$), respectively. For Tannin sheep, mean saliva volumes were 1.12, 1.56, and 2.43 L/d on P1, P2, and P3 ($P = 0.06$), respectively. As DMI was affected neither by the diet ($P = 0.93$) nor by period ($P = 0.95$), the ensalivation of the feed (mL of saliva/g of DMI) was also unaffected by the diet ($P = 0.50$) and period ($P = 0.21$; Table 1).

There were no differences ($P = 0.39$) in saliva production when goats were fed Tannin compared with goats in the Control group (Table 1). Furthermore, there was no treatment × period interaction observed ($P = 0.61$). The mean parotid saliva outputs for goats fed the Control diet were 1.65, 1.79, and 1.86 L/d ($P = 0.95$) for P1, P2, and P3, respectively. Corresponding parotid saliva outputs for goats fed the Tannin diet were 1.45, 2.32, and 2.50 L/d ($P = 0.20$) for P1, P2, and P3, respectively. Intake was not affected either by the diet ($P = 0.35$) or by period ($P = 0.35$), and consequently

Table 1. Parotid saliva production, ensalivation of feed, and DMI in sheep and goats fed the Control[1] or the Tannin[1] diet for 64 d

	Diet			Experimental period[2]				*P*-values		
Item	Control	Tannin	SEM	P1	P2	P3	SEM	Diet	Per[3]	Diet × Per
Sheep										
Parotid saliva production, L/d	2.14	1.71	0.288	1.59	1.84	2.34	0.271	0.31	0.14	0.30
Ensalivation of feed, mL/g DMI	3.51	2.81	0.736	2.58	3.05	3.86	0.613	0.50	0.21	0.50
DMI, g/d	642	654	97.2	638	657	649	75.1	0.93	0.95	0.15
Goats										
Parotid saliva production, L/d	1.77	2.09	0.260	1.55	2.05	2.18	0.313	0.39	0.37	0.61
Ensalivation of feed, mL/g DMI	2.51	2.65	0.417	2.45	2.41	2.88	0.426	0.81	0.58	0.62
DMI, g/d	762	845	59.9	730	891	790	76.0	0.35	0.35	0.97

[1]Control = alfalfa hay; Tannin = alfalfa hay supplemented with 50 g quebracho/kg DM.

[2]P1 = d 1 and 2; P2 = d 31 and d 32; P3 = d 61 and d 62.

[3]Per = experimental period.

ensalivation of the feed (mL of saliva/g of DMI) was unaffected by the diet ($P = 0.81$) and period ($P = 0.58$).

The data related to water intake, rumen osmotic pressure, rumen liquid kinetics, and calculated saliva flow for sheep are reported in Table 2. The inclusion of quebracho in the diet had no impact in water intake ($P = 0.98$), rumen OP ($P = 0.41$), rumen liquid volume ($P = 0.31$), liquid dilution rate ($P = 0.40$), rumen outflow ($P = 0.52$), estimated water flux ($P = 073$), and calculated total saliva flow ($P = 0.50$). Mean daily total saliva flow for Control sheep on P1, P2, and P3 was 7.64, 7.40, and 6.63 L, respectively. Corresponding values for Tannin sheep were 7.60, 9.83, and 7.21 L/d, respectively.

The data related to water intake, rumen osmotic pressure, rumen liquid kinetics, and calculated saliva flow for goats are reported in Table 3. Water intake increased ($P = 0.01$) from P1 to P3 and the rumen liquid volume increased accordingly. The rumen OP increased ($P = 0.01$) from P1 to P2 and as a consequence, there was a decrease ($P = 0.02$) in the estimated water flux through the rumen wall from P1 to P2. The calculated total saliva production in goats was not affected by feeding quebracho ($P = 0.97$) or by period ($P = 0.91$). Mean daily total saliva flow for Control goats on P1, P2, and P3 were 7.10, 8.19, and 7.41 L, respectively. Corresponding values for Tannin goats were 7.67, 7.30, and 7.81 L/d, respectively.

The composition of parotid saliva for Control- and Tannin-fed sheep is reported in Table 4. Salivary pH was decreased ($P = 0.03$) on P2 in comparison with P1 and P3 but was not affected ($P = 0.11$) by the diet. The OP of the saliva was not affected by the diet ($P = 0.13$) or period ($P = 0.54$). The concentrations of P, Mg, and Ca were unaffected ($P > 0.10$) either by the diet or by the period. The concentration of Na was greater ($P = 0.05$) and the concentration of K was reduced ($P = 0.04$) in sheep receiving the diet with quebracho in all periods. The urea concentration in the parotid saliva was similar ($P = 0.90$) for the Control and Tannin diets and also for P1, P2, and P3 ($P = 0.24$). However, there was a decrease in the urea concentration in the parotid saliva of sheep receiving the Control diet on P3 ($P = 0.02$), with no time effects in the sheep receiving the Tannin diet ($P = 0.21$). The protein content of the parotid saliva was unaffected ($P = 0.52$)

Table 2. Water intake, rumen liquid kinetics, and calculated saliva flow in sheep fed the Control[1] or the Tannin[1] diet for 64 d

	Diet			Experimental period[2]				*P*-values		
Item	Control	Tannin	SEM	P1	P2	P3	SEM	Diet	Per[3]	Diet × Per
Water intake										
Drinking water, L/d	2.51	2.49	0.544	2.44	2.31	2.75	0.446	0.98	0.63	0.76
Water in food, L/d	0.21	0.23	0.027	0.21	0.21	0.23	0.025	0.62	0.84	0.82
Osmotic pressure, mosmol/kg	207	222	11.76	222	213	208	10.59	0.41	0.44	0.85
Rumen liquid volume, L	5.42	6.89	0.964	5.22	6.14	7.10	0.841	0.31	0.14	0.99
Liquid dilution rate, proportion/h	0.065	0.059	0.004	0.070	0.065	0.050	0.0053	0.40	0.05	0.61
Rumen outflow, L/d	8.17	9.45	1.407	8.87	9.68	7.88	1.113	0.52	0.28	0.48
Estimated water flux, L/d	1.16	1.10	0.114	1.05	1.15	1.20	0.097	0.73	0.37	0.75
Calculated saliva flow, L/d	7.22	8.21	1.030	7.62	8.61	6.92	0.828	0.50	0.16	0.33

[1]Control = alfalfa hay; Tannin = alfalfa hay supplemented with 50 g quebracho/kg DM.

[2]P1 = d 4; P2 = d 34; P3 = d 64.

[3]Per = experimental period.

Table 3. Water intake, rumen liquid kinetics, and calculated saliva flow in goats fed the Control[1] or the Tannin[1] diet for 64 d

	Diet			Experimental period[2]				*P*-values		
Item	Control	Tannin	SEM	P1	P2	P3	SEM	Diet	Per[3]	Diet × Per
Water intake										
Drinking water, L/d	2.39	2.85	0.376	1.87[b]	2.37[b]	3.62[a]	0.384	0.40	0.01	0.63
Water in food, L/d	0.25	0.27	0.024	0.27	0.27	0.24	0.018	0.72	0.08	0.42
Osmotic pressure, mosmol/kg	254	241	6.44	231[b]	261[a]	250[ab]	6.21	0.19	0.005	0.50
Rumen liquid volume, L	4.81	5.12	1.092	3.16[b]	5.69[a]	6.04[a]	0.939	0.84	0.02	0.90
Liquid dilution rate, proportion/h	0.087	0.101	0.0146	0.115	0.083	0.082	0.0137	0.50	0.10	0.62
Rumen outflow, L/d	9.15	9.54	0.750	8.36	9.40	10.28	0.726	0.72	0.14	0.54
Estimated water flux, L/d	0.76	0. 92	0.056	0.98[a]	0.73[b]	0.81[ab]	0.057	0.08	0.02	0.60
Calculated saliva flow, L/d	7.57	7.59	0.525	7.38	7.74	7.61	0.608	0.97	0.91	0.62

[a,b]Within a row, means for the experimental period without a common superscript differ ($P < 0.05$).

[1]Control = alfalfa hay; Tannin = alfalfa hay supplemented with 50 g quebracho/kg DM.

[2]P1 = d 4; P2 = d 34; P3 = d 64.

[3]Per = experimental period.

by diet; however, there was a decrease in protein content observed in P3 when compared with P1 ($P = 0.02$).

The data related to the composition of the goat parotid saliva for the 2 diets and the 3 periods are reported in Table 5. The pH of the saliva was decreased ($P = 0.05$) when goats received the Control diet and greater on P1 than on P2 ($P < 0.001$) and P3 ($P < 0.001$) without differences between P2 and P3 ($P = 0.98$). The OP of the goat saliva was not affected by the diet ($P = 0.34$) or period ($P = 0.10$). The concentration of P in the parotid saliva was unaffected by the diet ($P = 0.32$) whereas there was an increase from P1 to P3 ($P = 0.01$). The concentration of Ca was unaffected by the diet ($P = 0.82$) but increased from P1 to P2 ($P = 0.02$) and also from P1 to P3 ($P = 0.05$) without differences between P2 and P3 ($P = 0.51$). There was an increase in the concentration of Na in saliva from P1 to P2 ($P = 0.01$) and P3 ($P = 0.01$) without differences ($P = 0.35$) between diets. The concentrations of K, Mg, and urea in the saliva of goats were not affected ($P > 0.10$) by the diet or the period. The protein content of the parotid saliva was unaffected by the diet ($P = 0.21$) and was greater on P3 than on P2 ($P = 0.01$) and P1 ($P = 0.02$).

DISCUSSION

The adaptation of the animals to the experimental conditions, the ingestion of quebracho, and the insertion of the collection tube in the parotid duct were satisfactory, taking into account that there was not a decrease in the amount of food consumed. In our study, animals were fed either alfalfa hay or alfalfa hay supplemented with quebracho at a dose (50 g quebracho/kg DM) that may cause changes in the digestive feed use without affecting DMI or causing malaise or toxic effect (Dawson et al., 1999; Villalba et al., 2002; Hervas et al., 2003).

Although it is widely recognized that saliva plays a key role in a number of digestive processes (Carter and

Table 4. Parotid saliva composition in sheep fed the Control[1] or the Tannin[1] diet for 64 d

	Diet			Experimental period[2]				*P*-values		
Item	Control	Tannin	SEM	P1	P2	P3	SEM	Diet	Per[3]	Diet × Per
pH	8.77	8.91	0.053	8.97[a]	8.66[b]	8.90[a]	0.070	0.11	0.03	0.57
Osmotic pressure, mosmol/kg	251	283	13.7	278	252	271	18.0	0.13	0.54	0.43
P, mmol/L	20.4	26.1	2.36	26.9	21.5	21.5	2.83	0.13	0.21	0.97
Mg, mmol/L	0.09	0.10	0.013	0.11	0.08	0.09	0.017	0.48	0.50	0.28
Ca, mmol/L	0.17	0.22	0.044	0.20	0.14	0.24	0.058	0.45	0.52	0.41
Na, mmol/L	154	195	12.6	185	160	179	16.5	0.05	0.52	0.28
K, mmol/L	21.1	10.5	2.85	18.8	18.1	10.4	3.31	0.04	0.12	0.14
Urea, mmol/L	3.08	3.00	0.410	2.88	3.55	2.69	0.419	0.90	0.24	0.02
Proteins, g/L[4]	0.109 (0.064–0.186)	0.089 (0.055–0.145)		0.177[a] (0.106–0.297)	0.113[ab] (0.058–0.220)	0.048[b] (0.026–0.087)		0.52	0.02	0.16

[a,b]Within a row, means for the experimental period without a common superscript differ ($P < 0.05$).

[1]Control = alfalfa hay; Tannin = alfalfa hay supplemented with 50 g quebracho/kg DM.

[2]P1 = d 1 and 2; P2 = d 31 and 32; P3 = d 61 and 62.

[3]Per = experimental period.

[4]Protein contents were log-transformed, so confident limits (in parentheses) are presented instead of SEM.

Grovum, 1990; Humphrey and Williamson, 2001), there are few studies reporting quantitative measurements of saliva output and composition in sheep and goats. In this study, parotid saliva was measured in both species using a unilateral model, and an alternative approach for the indirect estimation of total saliva production, based on water kinetics and balance in the rumen, was proposed. The calculation of salivary secretion based on water balance in the rumen involves quantification of inputs into the forestomachs (drinking and feed water) and estimation of water outputs from the rumen either by absorption through the rumen wall or outflow to the lower tract. Liquid dosed with the marker or withdrawn with samples is also accounted for in the calculation but not other possible losses (water spoiled from the drinker or ruminal fluid leakage from the cannula) that can be considered of marginal significance. Liquid outflow from the rumen can be determined using markers, and net water absorption is estimated from the OP of the ruminal contents (López et al., 1994). This latter approximation may determine the accuracy of the estimate of saliva secretion. However, the transepithelial net flux of water is normally small (Von Engelhardt, 1970), contributing to approximately 4 to 19% of salivary secretion (Duric et al., 1994), thus having a small impact on the estimate of salivary flow. In fact, our values of daily saliva secretion were in the range of those measured with direct methods (Kay 1960, 1966) in sheep fed forage diets at similar levels of intake to those in the present study.

Binding dietary tannins with salivary proteins has been suggested as a means to alleviate the adverse effects of the ingestion of condensed tannins by herbivores (Shimada, 2006; Waghorn, 2008), but there is scarce published information examining if this adaptive mechanism can be induced or developed in sheep and goats. Therefore, the present work was designed to investigate, under controlled experimental conditions, whether saliva production and composition could be affected in response to the regular and persistent ingestion of quebracho tannins in these ruminant species.

The lack of effects of the ingestion of quebracho and the period on the amount of parotid saliva produced by sheep and goats would indicate that under the conditions of this study, there was no response in salivation to the ingestion of quebracho tannins in these ruminant species. However, the daily amount of parotid saliva produced throughout the experiment was relatively steady when the animals were fed the Control diet and noticeably fluctuating with the Tannin diet, both in sheep and goats. Thus, sheep fed the Tannin diet produced 55, 73, and 107% of the amount of saliva recorded in sheep fed the Control diet on P1, P2, or P3, respectively. Corresponding values in goats were 88, 130, and 134% on P1, P2, or P3, respectively.

The ability of mammals to cope with the intake of tannins is associated with some adaptations in the digestive system. One of the first physiological responses occurs in the oral cavity, changing the volume and composition of saliva secreted (Mehansho et al., 1987; Makkar, 2003; Mueller-Harvey, 2006). In ruminants, a distinctive development of the parotid gland has been observed for the different feeding habits (i.e., grazers vs. browsers). The relative parotid mass was 0.18 to 0.22% of body mass in browsers and 0.05 to 0.07% in grazers (Hofmann, 1989). However, the effect of this difference in size on the amount of saliva being produced is unclear (Hofmann et al., 2008). Sheep and goats are capable of eating diets with substantial amounts of tannin browse, but it is well documented that goats consume more browse (Estell, 2010) and use tannin-rich foods more efficiently than sheep (Landau et al., 2000; Villalba et al., 2002). Some results indicate that goats have parotid

Table 5. Parotid saliva composition in goats fed the Control[1] or the Tannin[1] diet for 64 d

Item	Diet			Experimental period[2]				*P*-values		
	Control	Tannin	SEM	P1	P2	P3	SEM	Diet	Per[3]	Diet × Per
pH	7.92	8.04	0.032	8.26[a]	7.84[b]	7.85[b]	0.040	0.05	<0.001	0.02
Osmotic pressure, mosmol/kg	200	169	22.2	151	181	222	24.4	0.34	0.10	0.31
P, mmol/L	35.2	30.4	3.18	23.9[b]	35.2[a]	39.3[a]	3.99	0.32	0.03	0.16
Mg, mmol/L	0.07	0.08	0.014	0.07	0.09	0.08	0.018	0.67	0.80	0.11
Ca, mmol/L	0.15	0.15	0.017	0.10[b]	0.18[a]	0.16[a]	0.021	0.82	0.04	0.28
Na, mmol/L	153	134	12.9	96[b]	170[a]	166[a]	15.9	0.35	0.01	0.15
K, mmol/L	22.7	19.6	4.81	10.8	25.9	26.7	5.44	0.67	0.07	0.71
Urea, mmol/L	1.78	2.38	0.363	2.12	1.98	2.14	0.386	0.28	0.94	0.53
Proteins, g/L[4]	0.028 (0.013–0.059)	0.050 (0.025–0.102)		0.024[b] (0.011–0.055)	0.020[b] (0.008–0.048)	0.109[a] (0.051–0.234)		0.21	0.02	0.34

[a,b]Within a row, means for the experimental period without a common superscript differ ($P < 0.05$)

[1]Control = alfalfa hay; Tannin = alfalfa hay supplemented with 50 g quebracho/kg DM.

[2] P1 = d 1 and d 2; P2 = d 31 and d 32; P3 = d 61 and d 62.

[3]Per = experimental period.

[4]Protein contents were log-transformed, so confident limits (in parentheses) are presented instead of SEM.

glands of a greater size relative to BW (Vaithiyanathan et al., 2001) and produce more parotid saliva than sheep in some circumstances (Seth et al., 1976, Domingue et al., 1991). Based on these comparative studies, it could be postulated that parotid saliva would be a medium to counteract the negative effects of tannins in goats but not in sheep. In the current experiments, there was not an effect of the inclusion of quebracho in the diet on parotid saliva production in sheep or in goats. However, the differences in the parotid saliva output in P1 (immediately after the administration of quebracho) between Control and Tannin animals were considerably greater in sheep compared with goats. Furthermore, the ratio between the parotid saliva production by the animals receiving the Tannin diet and the animals fed the Control diet followed a somehow different pattern in sheep and in goats. Both results could indicate a better ability of the parotid gland of goats than the parotid gland of sheep in responding to the ingestion of condensed tannins (Marsh et al., 2006).

The proportion of total saliva secreted by the parotid gland, calculated assuming the same amount of saliva being produced for both parotids, was similar to the values reported by Kay (1960, 1966). This ratio (parotid saliva:total saliva) was unaffected by diet in sheep or goats. However, there was an increase in this ratio in P3 when compared with P1 and P2 in sheep; the increase was most evident in sheep fed the Tannin diet. This evolution in the contribution of parotid saliva to the total saliva seems to indicate that the parotid gland is affected by tannins to a greater extent than the other salivary glands. The possibility that the different salivary gland complexes might compensate for each other (Hofmann et al., 2008) could be an explanation. On the other hand, the effect of condensed tannins depressing parotid secretion would be more persistent in sheep than in goats.

Studies on rumen fluid kinetics have shown differences between ruminant species, with decreased retention times of liquid digesta for grazers in comparison with browsers (Lechner et al., 2010). These differences were associated with frothy rumen contents in browsers versus a more stratified ruminal digesta in grazers, partly determined by more viscous saliva in browsers (Clauss et al., 2008). Clauss et al. (2010) stated that the linkage between the ruminant digestion type and dietary niches requires more detailed investigation. The results of this study indicate that the inclusion of quebracho in the diet (i.e., moving toward a browser diet) does not affect any of the parameters of the water kinetics in the rumen in both ruminant species. It is difficult to find a physiological explanation for the observed time effects after a regular ingestion of condensed tannins on ruminal water balance although these time effects could simply be due to changes in water intake, probably associated with small variations in the room temperature. There are no data in the literature measuring the direct effect of tannins on ruminal water balance although Silanikove et al. (2001) have reported an increase in rumen volume with no changes in the liquid digesta retention time in the rumen of goats given polyethylene glycol as a neutralizing agent of tannins.

Mineral contents, pH, and OP, which were mostly unaffected by the ingestion of quebracho, were within the range of the values published in the literature (Young and Schneyer, 1981; Cook, 1995). It is well known that the concentrations of Na and K in parotid saliva vary according to the secretion rate (Young and Schneyer, 1981; Cook, 1995). Although we did not find differences in the amount of parotid saliva produced either by sheep or goats, small differences in the secretion rate could be responsible for the changes observed in the concentrations of Na and K in parotid saliva in both experiments.

The protein content of sheep saliva was greater and that of goat saliva less than the values reported by González and Grovum (1994) but in both cases were within the range of values published recently by Lamy et al. (2008). These authors reported a strong similarity between the electrophoretic profiles of sheep and goat parotid saliva proteins. Salivary proteins that are rich in proline or histatins have been described in different animal species consuming tannin-rich diets (McArthur et al., 1995, Fickel et al., 1998; Shimada, 2006). These types of proteins have not been found in domestic ruminants such as cows, sheep, and goats (Austin et al., 1989; Makkar and Becker, 1998). In the present study, the concentration of these proteins were not determined, but TP content of the parotid saliva was marginal and not affected by the ingestion of quebracho condensed tannins, suggesting that this adaptation mechanism was not developed in sheep and goats. These findings are in agreement with Hanovice-Ziony et al. (2010), who concluded that the ingestion of tannins does not imply that salivary tannin binding is a mechanism used to counteract the deleterious effects of tannins in goats. On the contrary, Alonso-Diaz et al. (2010) reported some evidence of the presence of tannin-binding salivary proteins in tropical goats and hair sheep.

Conclusion

This study suggests that feeding quebracho tannins at a concentration of 50 g/kg diet for 64 d does not appear to affect salivation (parotid and total saliva) in sheep and goats. Nevertheless, the evolution in the ratio between the amounts of parotid saliva produced by the animals fed the Control diet and the Tannin diet seems to indicate that goats might increase the production of parotid saliva in response to the steady ingestion of quebracho

resulting in a better potential ability in dealing with the consumption of condensed tannins. This work also demonstrates and validates an alternative approach for the indirect estimation of total saliva production, based on water kinetics and balance in the rumen, and provides data on parotid saliva composition in sheep and goats.

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