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Depression of rumen ammonia and blood urea by quebracho tannin-containing supplements fed after high-nitrogen diets with no evidence of self-regulation of tannin intake by sheep

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ABSTRACT

The aim of this study was to assess whether sheep enhance nitrogen use in response to tannin supplementation, and if they increase preference for tannin-rich supplements when consuming diets high in rumen-degradable protein. In Experiment 1 twenty four Corriedale crossbred wethers (10 months of age; 41 ± 2.1 kg) were randomly divided into two groups (n = 12/group). Group 1 received a high-protein basal diet of high rumen-degradable protein ("HP"), whereas the other group received a low-protein basal diet of low rumen-degradable protein ("LP"). During Period 1 (12 days) half of the animals from each group received a wheat bran supplement containing 11% quebracho tannins (T) and the other half had the same supplement but without tannins (NT). Wethers fed supplements T or NT during Period 1 received NT or T, respectively, during Period 2. Rumen fluid and jugular blood samples were collected on the last day of each period to determine ruminal ammonia nitrogen and blood urea nitrogen (BUN) concentration, respectively. Experiment 2 was similar to Experiment 1, except that a new set of wethers was used (11 months of age; 41 ± 2.4 kg), oat straw replaced wheat bran, and only BUN was determined. After Period 2 of Experiment 2, all animals had a simultaneous offer of T and NT and preference for T was estimated for 3 days. In Experiment 1, wethers in HP consumed greater (P < 0.05) amounts of basal diet than wethers in the LP during Period 1. Wethers exposed to HP displayed lower values of BUN and ruminal ammonia nitrogen when consuming T than when ingesting NT (Periods 1 and 2, respectively; P < 0.01). In Experiment 2, wethers under the HP diet and supplemented with T consumed the greatest amounts of basal diet (Period 1; P < 0.05). Intake of tannin was higher in LP than in HP (P < 0.05) and wethers exposed to HP displayed lower values of BUN when consuming T than when ingesting NT (Period 2; P < 0.05). Preference for and intake of the tannin-containing food tended (P = 0.11) to be greater for wethers exposed to HP than for wethers under LP during the last day of testing. Tannin supplements have the potential to reduce rumen ammonia nitrogen and BUN in sheep, even when fed after ingestion of high-N diets and in the form of low-quality supplements.

1. Introduction

The traditional view of plant secondary compounds as defences against herbivory (Rosenthal and Janzen, 1979; Cheeke and Shull, 1985) has expanded in recent times due to the fact that some of these compounds in moderate

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amounts have positive effects on herbivores. For instance, by eating plants with tannins, a group of phytochemicals (proanthocyanidins), herbivores reduce internal parasites (Min and Hart, 2003). Tannins also alleviate bloat by binding to proteins in the rumen (Waghorn, 1990) and they reduce methane emissions (Woodward et al., 2004). By making dietary protein unavailable for ruminal digestion until it reaches the more acidic abomasum and small intestines, modest amounts of tannins improve the protein nutrition of ruminants (Barry et al., 2001; Min et al., 2005). This high quality bypass-protein enhances immune responses (Niezen et al., 2002; Min et al., 2004) and improves reproductive efficiency (Min et al., 2001). In addition, a reduced protein digestion in the rumen decreases the rate of ammonia production, a potentially toxic chemical detoxified in the liver (Chalupa et al., 1970) which represents a metabolic cost to the host (Parker et al., 1995).

As a result of the aforementioned benefits on protein metabolism, commercial quebracho tannins have been proposed as an additive for protecting rumen-degradable protein in feeds (Frutos et al., 2000; Getachew et al., 2008). In addition to its use as an additive, tannin in supplements could also represent a useful alternative for improving nitrogen use in ruminants. Animals consuming pastures or rations with high concentrations of rumen-degradable protein may benefit from tannin-rich supplements. For instance, quebracho tannins have been supplied dissolved in water (Komolong et al., 2001; Kronberg, 2008) or in shrubs (Acacia) before and after feeding protein concentrates (Ben Salem et al., 2005).

Forcing animals into ingesting tannin-containing rations may have negative postingestive consequences, as at certain doses tannins can depress voluntary intake, digestive efficiency and animal productivity (Hervas et al., 2003). An alternative, which has not been explored, is the ability of herbivores to self-regulate tannin ingestion as a function of dietary nitrogen content. Recent evidence suggests ruminants are able to select substances that rectify states of malaise, i.e., they self-medicate. As an example, sheep self-select supplements containing polyethylene glycol in response to a tannin challenge (Provenza et al., 2000; Villalba and Provenza, 2002), increase intake of antiacids in response to rumen acidosis (Phy and Provenza, 1998), and parasitized sheep increase preference for tannin-containing rations relative to non-parasitized animals (Villalba et al., 2010). However, no information is available on the effectiveness of tannin-rich supplements at enhancing nitrogen metabolism or on the ability of ruminants to self-select tannin-rich supplements when fed basal diets high in protein. Thus, the objectives of the present study were to assess whether (1) tannin supplementation reduces rumen ammonia N and blood urea N (BUN) in sheep fed different levels of protein with their diets (Experiment 1), and (2) sheep increase preference for tannin-rich supplements, even of low nutritional quality, when consuming diets high in rumen-degradable protein (Experiment 2).

2. Materials and methods

The study was conducted at the Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), CONICET and Universidad Nacional del Sur, Bahía Blanca (Lat. 38°47′ Lo 62°37′, Argentina). Handling of animals and experimental protocols in the present study met the code of practice for the care and handling of farm animals used in research at Universidad Nacional del Sur, which adheres to the ASAB/ABS Guidelines for the Use of Animals in Research (2006).

Before beginning each feeding experiment food samples ($n = 10$) were oven dried at 60 °C to a constant weight, ground to pass through a 1 mm screen, and analyzed for crude protein (CP) by the Kjeldahl procedure (AOAC, 1990) and neutral detergent fibre (NDF) by the detergent system (Goering and Van Soest, 1970). Rumen-degradable protein (RDP) was calculated from equations provided by Sniffen et al. (1992), whereas metabolizable energy (ME) was estimated according NRC (1985).

2.1. Experiment 1

2.1.1. Adaptation to the basal diets

Twenty four Corriedale crossbred wethers (10 months of age; 41 ± 2.1 kg BW, mean ± 1 SD, respectively) were penned outdoors under a protective roof in individual, adjacent pens measuring 1.5 m × 1.5 m. The floor of the pens was slatted and raised 0.25 m. Wethers were randomly divided into two groups. One group ($n = 12$), “HP”, received a high-protein basal diet (22% CP) of high rumen-degradable protein (17% RDP): 47% sunflower meal pellets, 20% corn grain, 20% alfalfa hay, 11% oat straw, 2% vitamins and minerals. The other group ($n = 12$), “LP”, received a low-protein basal diet (11% CP) of low rumen-degradable protein (8% RDP): 47% corn grain, 30% alfalfa hay, 21% oat straw, 2% vitamins and minerals. Basal diets were isocaloric (2.4 Mcal ME kg^{-1}). They were offered daily between 09:30 and 11:00 at 2.4% BW per day. Animals were familiarized to their respective basal diets for 14 consecutive days.

2.1.2. Exposure to diets and supplements

Immediately after familiarization, in Period 1 (14 days) all animals were offered their respective basal diets from 09:30 to 11:00. Refusals were collected and weighed. At 12:00 six randomly selected animals from each group (HP-T; LP-T) received in their respective pens a supplement containing tannins (T): 89% wheat bran (4–6 mm particle size) + 11% quebracho tannin extract (Unitan SAICA, Chaco, Argentina). The other half ($n = 6$; HP-NT; LP-NT) had the same supplement but without tannins (NT, placebo). Tannin-containing supplements were offered in quantities (400–500 g) that represented a concentration of tannins of 4% of the diet consumed. Supplements were removed at 15:00. No other food was offered until the next day. Since wethers consumed the whole amount of supplement offered, intake data was not analyzed statistically. Period 1 lasted 12 consecutive days.

We decided to use quebracho tannins because of its high protein binding activity (Hagerman et al., 1992), despite their potential negative postingestive effects (Robbins et al., 1991; Dawson et al., 1999).

Immediately after Period 1, supplements were switched in Period 2 such that animals previously offered T received NT and vice versa during the same number of days and under the same experimental conditions.

2.1.3. Rumen ammonia N and BUN

Rumen fluid and jugular blood samples were collected on the last day of each period to determine ruminal ammonia and BUN concentration, respectively. Since our objective was to assess the impact of tannins consumed in a supplement when ammonia concentrations peak in the rumen environment, samples of rumen fluid were obtained through a stomach tube 2.5 h after feeding the basal diets. Previous research suggests concentrations of ammonia in the rumen peak around 2.5 h after consumption of diets of high rumen protein degradability (Van Soest, 1994; Atkinson et al., 2010). Samples (50 mL) of rumen fluid were strained through 4 layers of cheesecloth and mixed with 0.5 mL of sulfuric acid 7.2 N, centrifuged and prepared for ammonia determinations (AOAC, 1990). Jugular blood samples were obtained 4.5 h after feeding the basal diets. Serum urea typically peaks 1.5–2.0 h after rumen ammonia peaks (Gustafsson and Palmquist, 1993). Blood samples were allowed to clot for 30 min and serum was collected after centrifugation (2300 × g for 15 min at 4 °C). Serum was stored at −20 °C for later analysis of urea. Serum urea concentrations were determined by an enzymatic colorimetric method (kit laboratory Wiener S.A.I.C).

Table 1
Nutritional analyses of the high-protein basal diet of high rumen-degradable protein (HP), low-protein basal diet of low rumen-degradable protein (LP), and supplement given to wethers in Experiment 1 and Experiment 2.

Food	Experiment 1				Experiment 2			
	CP (%)	RDP[a] (%)	NDF (%)	ME[b] (Mcal kg^{-1})	CP (%)	RDP (%)	NDF (%)	ME (Mcal kg^{-1})
HP	21.5	17.1	33.5	2.4	19.2	15.5	34.1	2.3
LP	11.3	8.4	34.2	2.4	11.8	7.5	34.5	2.3
Supplement[c]	16.6	13.6	31.1	2.3	4.5	0.9	76.4	1.6

CP, crude protein; RDP, rumen degradable protein; NDF, neutral detergent fibre; ME, metabolizable energy. Experiment 2 oat straw (85%) and quebracho tannins (15%).

[a] Rumen degradable protein was calculated from equations provided by Sniffen et al. (1992).

[b] Metabolizable energy was estimated according NRC (1985).

[c] Supplement in Experiment 1 was wheat bran (89%) and quebracho tannins (11%), and in Experiment 2 oat straw (85%) and quebracho tannins (15%).

2.2. Experiment 2

2.2.1. Adaptation period

A different group of twenty four Corriedale crossbred wethers (11 months of age; 41 ± 2.4 kg BW, mean ± 1 SD, respectively) were housed in the same experimental conditions described for Experiment 1. Wethers were randomly divided into two groups. One group (n = 12), "HP", received a high-protein basal diet (19% CP, AOAC, 1990) of high rumen-degradable protein (16% RDP): 56% sunflower meal pellets, 23% corn grain, 19% alfalfa hay, 2% vitamins and minerals. We speculated that the high concentration of CP in Experiment 1 led to strong negative postingestive effects that attenuated the potential benefits of tannins. It has been shown that high concentrations of ammonia in the rumen can trigger food aversions (Kertz et al., 1982; Villalba and Provenza, 1997). Thus, during Experiment 2 the concentration of CP was reduced to increase the likelihood of animals experiencing mild instead of high levels of malaise due to the high concentrations of rumen-degradable protein in the diet.

The other group (n = 12), "LP", received a low-protein basal diet (12% CP) of low rumen-degradable protein (8% RDP): 4% sunflower pellets, 33% corn grain, 61% alfalfa hay, 2% vitamins and minerals. Basal diets were isocaloric (2.3 Mcal ME kg^{-1}). They were offered daily between 10:00 and 11:30 at 2.4% BW per day. Animals were familiarized to their respective basal diets for 5 consecutive days.

After familiarization to the diets, animals were familiarized to two supplements. Every day at 12:00 and after receiving their respective basal diets, half of the animals selected at random from each group (n = 6; HP-T; LP-T) received a supplement containing tannins (T): 85% ground oat straw (4 mm particle size) + 15% quebracho tannin extract (Unitan SAICA, Chaco, Argentina), whereas the other half (n = 6; HP-NT; LP-NT) had the same supplement but without tannins (NT, placebo). Familiarization with the supplements lasted 10 days.

2.2.2. Conditioning

All wethers continued to receive their respective basal diets and supplements. Refusals were collected and intake was measured during 15 consecutive days (Period 1). After this period, supplements were switched such that animals previously offered T received NT and vice versa during the same number of days and under the same basal diets (Period 2). Tannin was offered in quantities that represented approximately 4% of the total amount of feed consumed on a daily basis. Thus, the maximum amount of supplement offered was 500 g. Refusals were collected at 11:30 and 17:00 and weighed, and intake of basal diet and supplements were determined.

Jugular blood samples were collected on the last day of each period at 16:00, allowed to clot for 30 min and serum was collected after centrifugation (2300 × g for 15 min at 4 °C). Serum was stored at −20 °C for later analysis of urea. Serum urea concentrations were determined by an enzymatic colorimetric method (kit laboratory Wiener S.A.I.C).

2.2.3. Preference tests

After Period 2, all animals were fed their respective basal diets (HP or LP) from 10:00 to 11:30. Refusals were collected and then wethers had a simultaneous offer of the supplements T and NT for 30 min. Intake was determined for all feeds and no other feed was offered until the next day. Preference tests were carried out for three consecutive days.

We did not want to satiate the animals before preference tests. Thus, the exposure to the basal diet was restricted to 90 min. On the other hand, animals almost stopped eating the low-quality tannin-containing supplement after the 30 min period.

2.3. Statistical analysis

Basal diet intake (expressed as a per kg of BW basis), rumen ammonia N, and BUN (Experiment 1), feed intake of basal diet, supplements, tannins (expressed as a per kg of BW basis) and BUN (Experiment 2) were analyzed as a split-plot design with wethers nested within group (HP-T; LP-T, HP-NT; LP-NT). Day and period were the repeated measures. Intake of basal diets (Experiments 1 and 2) and supplements (Experiment 2) used in the analyses were those that represented stable values across consecutive days, when intake differences between days were within a ±10% range (7 days).

The statistical design for the analysis of variance during preference tests was a split-plot. Food intake and preference for the tannin-containing supplement ([intake of T supplement/intake of T + NT supplements] × 100) were the dependent variables. Preference values were arcsine transformed to meet assumptions of normality and homogeneity of variances. Group (HP; LP) was the between-animal factor, and animals were nested within group. Test supplement (T, NT) was the within-animal factor in the analysis and day was the repeated measure. Statistical analyses were computed using Infostat software (Di Rienzo et al., 2008).

3. Results

Table 1 shows the chemical composition of foods offered to sheep in Experiment 1 and Experiment 2.

3.1. Experiment 1

3.1.1. Intake of the basal diet

Wethers in HP consumed greater (P < 0.05) amounts of basal diet than wethers in the LP diet during Period 1. However, differences disappeared during Period 2 (P > 0.05) (Fig. 1).

3.1.2. Rumen ammonia N and BUN

Wethers exposed to HP showed greater values of rumen ammonia nitrogen than wethers consuming LP (P < 0.01). In addition, wethers under the HP diet displayed lower values of ruminal ammonia nitrogen when consuming the tannin-containing supplement than when ingesting the supplement without tannins during Period 2 (P < 0.01) (Fig. 2).

Wethers consuming the HP diets showed higher serum values of BUN than wethers exposed to the LP basal diet (P < 0.01). In addition, within the LP diet, no differences in BUN were detected between groups of wethers consuming supplements with or without tannins during

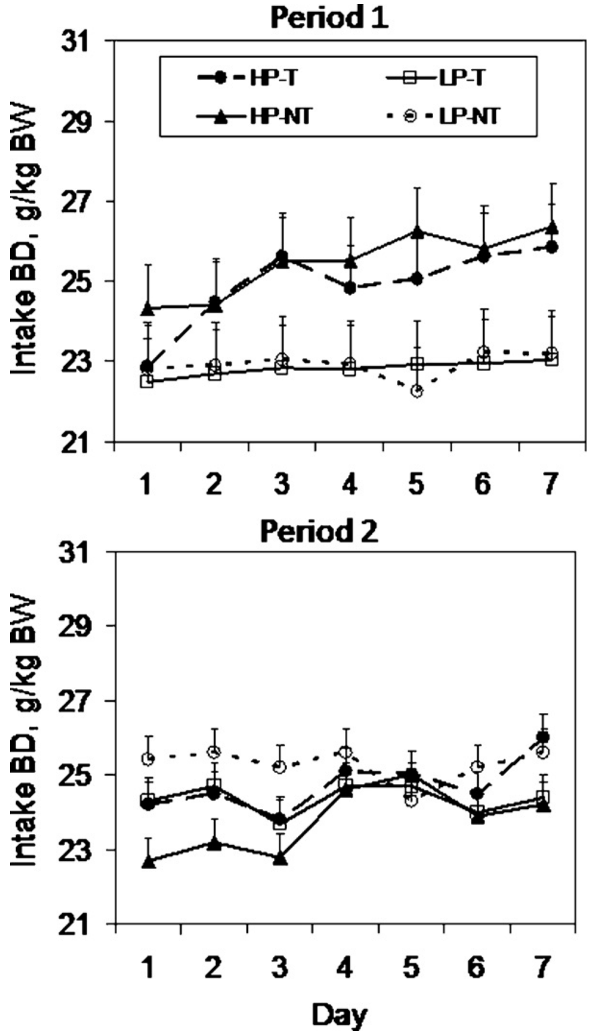

Fig. 1. Intake of basal diets (BD) by wethers during Experiment 1. Values are means of six animals. Bars represent + 1 SEM. HP, high-protein basal diet (22% CP) of high rumen-degradable protein (17% RDP). LP, low-protein basal diet (11% CP) of low rumen-degradable protein (8% RDP). Diets were supplemented with (T: 89% wheat bran + 11% quebracho tannin extract) or without (NT: placebo: wheat bran) tannins. Wethers fed supplements T or NT during Period 1 (12 days) received NT or T, respectively, during Period 2 (12 days).

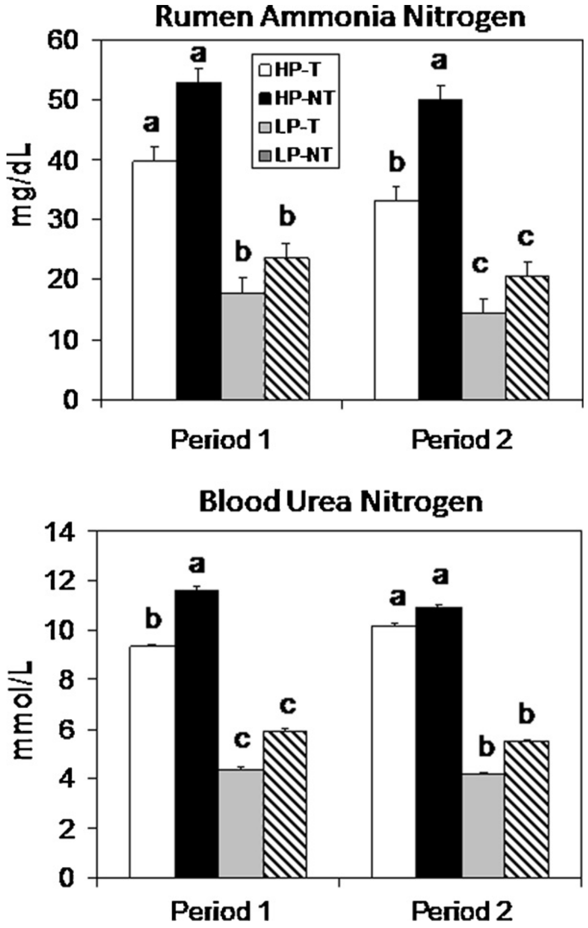

Fig. 2. Rumen ammonia nitrogen and blood urea nitrogen concentration in sheep during Experiment 1. Values, bars, basal diets (HP and LP) and supplements (T and NT) are as in Fig. 1. Rumen fluid and jugular blood samples were drawn on the last day of each period. Means within period not sharing a common letter (a, b, c) differ ($P < 0.05$).

Periods 1 and 2 ($P > 0.05$) (Fig. 2). In contrast, within the HP diet wethers consuming the tannin-containing supplement during Period 1 showed lower concentrations of BUN than wethers without tannin supplementation ($P < 0.01$). No supplement effect was found for wethers under the HP diet during Period 2 ($P > 0.10$) (Fig. 2).

3.2. Experiment 2

3.2.1. Intake of the basal diet

Intake of the basal diet did not differ between groups ($P > 0.10$; Fig. 3A and D). However, during Period 1 wethers under the HP diet and supplemented with tannins consumed the greatest amounts of basal diet ($P < 0.05$) (Fig. 3A).

3.2.2. Intake of supplements

Intakes of tannin-containing supplements were lower than intakes of supplements without tannin ($P < 0.01$). Wethers exposed to HP consumed lower amounts of supplement without tannin than wethers exposed to the LP basal diet ($P < 0.01$). Wethers fed the HP diet and tannins during Periods 1 and 2, and the LP diet and tannins during Period 2 consumed the lowest amounts of supplement ($P < 0.05$) (Fig. 3B and E).

3.2.3. Intake of tannins

For wethers offered tannin-containing supplements, intake of tannin was higher in LP than in HP ($P < 0.05$), attributed to marked differences in intake of tannin-containing feeds during Period 1 (Fig. 3C and F).

3.2.4. BUN

Wethers consuming the HP diets showed higher serum values of BUN than wethers exposed to the LP basal diet ($P < 0.05$). Within the LP diet, no differences in BUN were detected between groups of wethers consuming supplements with or without tannins ($P > 0.10$). Likewise, no

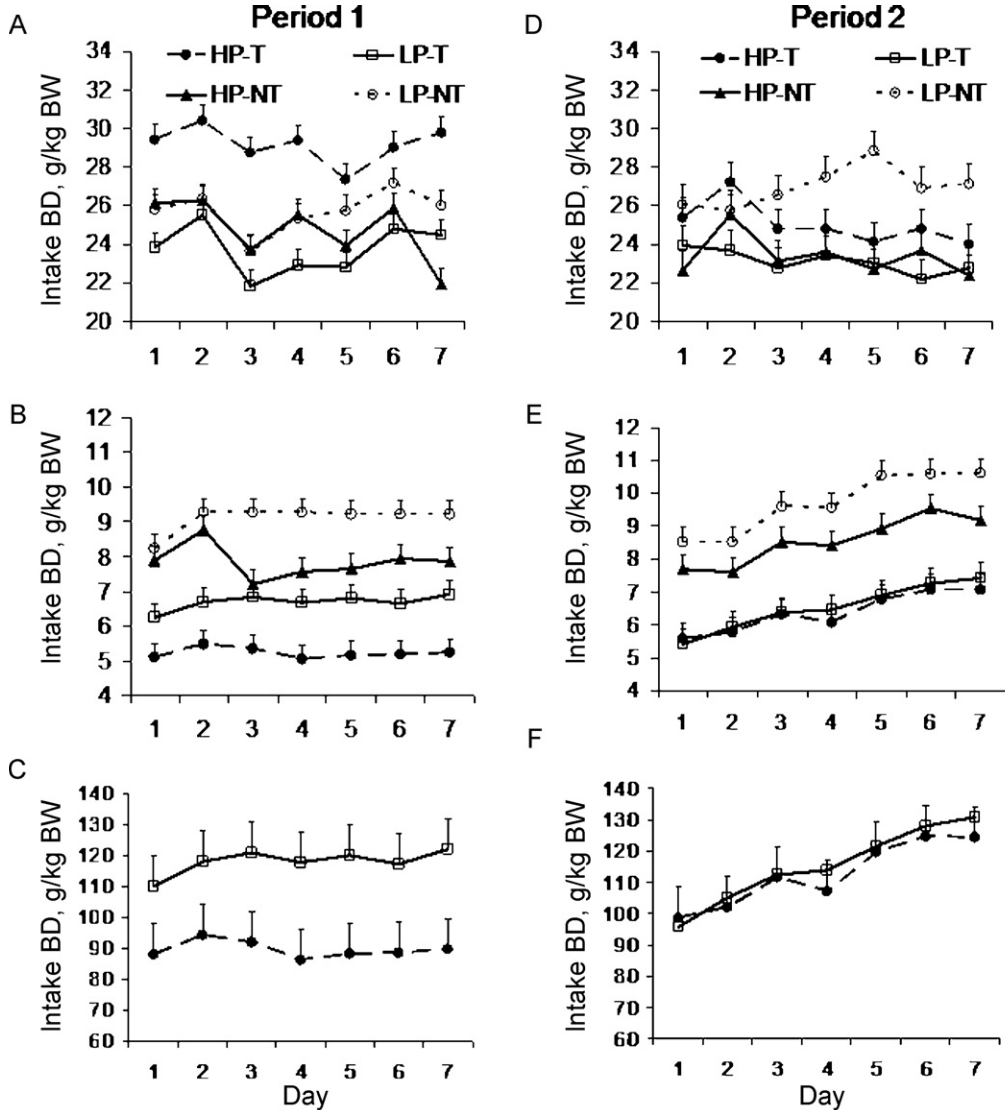

Fig. 3. Intake of: A/D – basal diets (BD), B/E – supplements, and C/F – tannins by wethers during Experiment 2. Values and bars are as in Fig. 1. HP, high-protein basal diet (19% CP) of high rumen-degradable protein (16% RDP). LP, low-protein basal diet (12% CP) of low rumen-degradable protein (8% RDP). Diets were supplemented with (T: 85% ground oat straw + 15% quebracho tannin extract) or without (NT: placebo: oat straw) tannins. Wethers fed supplements T or NT during Period 1 (15 days) received NT or T, respectively, during Period 2 (15 days).

supplement effect was found for wethers under the HP diet during Period 1 ($P > 0.10$). However, wethers consuming the HP diets during Period 2 showed lower concentrations of BUN when consuming tannins than when consuming the supplement without tannins (period effect; $P < 0.05$; Fig. 4).

3.2.5. Preference tests

No differences between basal diets were detected in preference for and intake of the tannin-containing diet for Days 1 ($P = 0.42$; 0.55) and 2 ($P = 0.29$; 0.50, respectively) of testing. In contrast, preference for and intake of the tannin-containing food tended ($P = 0.11$) to be greater for wethers exposed to the HP diet than for wethers under the LP diet (Fig. 5A and B).

4. Discussion

4.1. Tannins in diets vs. supplements

It is well known that at certain doses plant secondary metabolites (PSM) negatively impact animal cells, tissues, and metabolic processes (Cheeke and Shull, 1985; Osweiler et al., 1985; Cheeke, 1988). For instance, tannins eaten in too large amounts reduce the digestibility of plants and can be toxic (Pritchard et al., 1992; Hervas et al., 2003). However, at appropriate doses, PSM are potentially beneficial. Tannins in moderate concentrations exert positive effects on protein metabolism in ruminants, reducing rumen degradation of dietary protein, and increasing absorption of AA in the small intestine (Barry and McNabb, 1999).

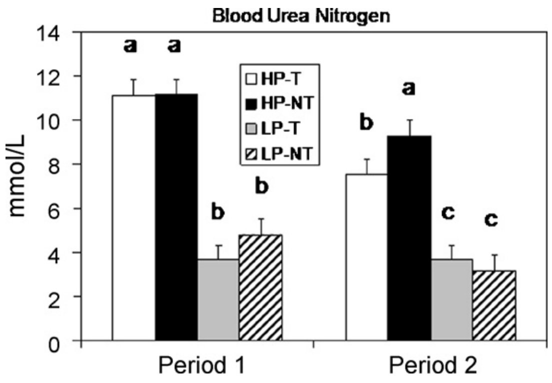

Fig. 4. Blood urea nitrogen concentration in sheep during Experiment 2. Values, bars, basal diets (HP and LP) and supplements (T and NT) are as in Fig. 3. Rumen fluid and jugular blood samples were drawn on the last day of each period. Means within period not sharing a common letter (a, b, c) differ ($P < 0.05$).

Dietary bypass-protein enhances immune responses and increases resistance to gastrointestinal nematodes (Niezen et al., 2002; Min et al., 2004).

As a consequence of the aforementioned benefits, commercial quebracho tannin extracts have been proposed as a useful feed additive for protecting protein in high-protein feeds against rumen degradation (Frutos et al., 2000). For livestock in confinement, obtaining adequate intake of tannins is possible by mixing tannins with feeds. This approach has received the most attention (Getachew et al., 2000; Turne and Neel, 2003) although when present in a mixed ration, even at low concentrations, tannins may continue exerting disadvantageous effects on nutrient intake and digestion (Provenza et al., 1990; Hervas et al., 2003). Moreover, ingestion of tannin-containing basal diets may vary substantially among individuals due to marked differences in food intake and tolerance to PSM which depend in part on variations in how animals are built morphologically and how they function physiologically (Provenza et al., 2003).

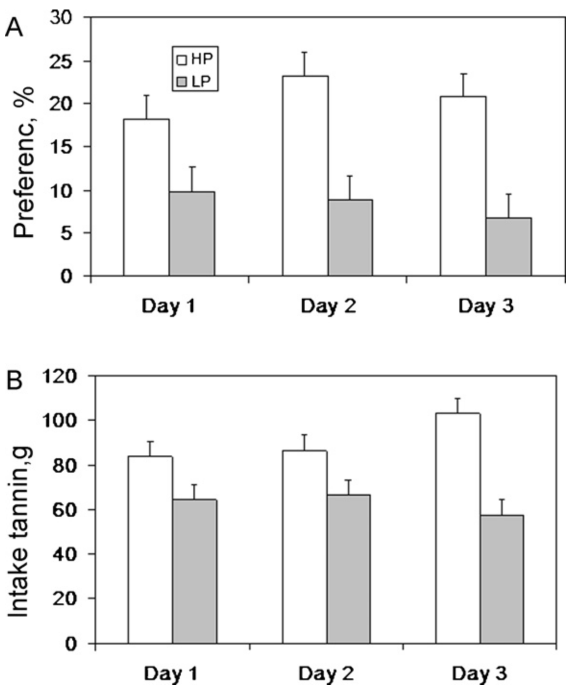

Fig. 5. Preference for the tannin-containing supplement (A) and intake of tannin (B) in preference tests during Experiment 2. Values, bars, basal diets (HP and LP) and supplements (T and NT) are as in Fig. 3.

Offering tannin-containing supplements may represent an alternative to overcome reductions in basal diet intake and individual variation. Moreover, tannin supplementation may represent a useful strategy for enhancing N use in animals grazing high N-containing pastures (Kronberg, 2008). Nevertheless, studies exploring the effects of tannin supplementation on nitrogen use by ruminants are scarce. Our study shows that sheep can consume low-quality tannin-rich supplements even after consuming a high-protein basal diet. Lambs could have completely avoided the supplement due to its low-quality and tannins content. Nevertheless, they ingested such type of supplement and in amounts that effectively attenuated rumen ammonia and concentrations of BUN. This suggests tannin-containing hay or pasture of low quality may enhance nitrogen retention of high-protein rations or forages by sheep. Previous studies have provided small ruminants with tannin-containing supplements, but of higher quality and before feeding the basal diet (e.g., Turne and Neel, 2003). Other studies have offered to sheep and cattle quebracho tannin dissolved in water (Kronberg, 2008). Supplementing sheep with tannin-rich shrubs has also increased efficiency of nitrogen use in ruminants fed soybean meal (Ben Salem et al., 2005).

Nitrogen-rich feeds reduce food intake as a consequence of excess rumen ammonia nitrogen and build-up of urea nitrogen in blood, leading to toxicity in ruminants (Kertz et al., 1982). Sheep develop aversions to feeds paired with intraruminal infusions of urea (Villalba and Provenza, 1997). Our results show that tannin supplementation reduces concentrations of rumen ammonia nitrogen (Experiment 1) and BUN (Experiments 1 and 2), suggesting a positive effect of tannin supplementation on protein metabolism and animal health.

4.2. Tannins in supplements and basal diet intake

During Experiment 2, wethers offered the high-protein diet consumed the greatest amounts of basal diet when supplemented with tannins. It is likely that tannins attenuated the negative effects of excess nitrogen in the wethers' internal environment allowing for an increased consumption of the basal diet. In agreement with this, the concentration of BUN did not differ between groups of wethers consuming the HP basal diet, even when wethers supplemented with tannins consumed greater amounts of HP basal diet than wethers supplemented with straw without tannins (Experiment 2).

4.3. Self-selection of tannin-containing supplements

Food selection in ruminants can be interpreted as the quest for substances in the external environment that provide a homeostatic benefit to the internal environment (Villalba and Provenza, 2007). Consistent with this, sheep ingest compounds such as sodium bicarbonate (Phy and Provenza, 1998) and bentonite (Villalba et al., 2006a) to alleviate acidosis. Sheep also increase intake of medicinal substances such as polyethylene glycol (PEG), a polymer that attenuates the aversive effects of tannins, as tannin concentrations in their diet increase (Provenza et al., 2000). They discriminate the medicinal effects of PEG from "non-medicinal" substances by selectively increasing intake of PEG after eating a meal high in tannins (Villalba and Provenza, 2001). Multiple malaise-medicine associations in herbivores are also possible. Sheep had to learn about the benefits of specific medicines at alleviating the effects of illness-inducing foods in order to choose the right treatment. They selected an anti-acid (sodium bentonite) after ingesting a meal that causes ruminal acidosis, and they preferred substances that neutralize the effects of tannins (PEG) and oxalic acid (dicalcium phosphate) after ingesting meals with these PSM (Villalba et al., 2006a). Control animals which were not given the opportunity to learn about the association of medicine-malaise (e.g., they consumed the medicine without experiencing malaise) did not choose the right medicine and the pattern of medicine selection did not change after consuming different illness-inducing foods (Villalba et al., 2006a). Sheep also increase intake and preference for tannin-containing foods when they are infected with endoparasites relative to when parasitic loads are not present (Villalba et al., 2010).

This study was the first at attempting to explore self-selection of tannin-containing feeds in ruminants fed different levels of protein with their diets. Wethers under a high-protein diet tended to show greater preferences for the tannin-containing supplement than wethers fed the low-protein diet. However, differences between groups were not significant even when tannin supplementation likely improved protein use by wethers, as suggested by rumen ammonia N and BUN values.

While research on self-selection of nutrients and medicines by herbivores is increasing (Villalba and Landau, 2011), the information available is sometimes anecdotal and equivocal (Clayton and Wolfe, 1993; Lozano, 1998). Some observations and surveys that are consistent with the self-medication hypothesis (e.g., Gradé et al., 2009) do not establish cause and effect relationships (Lozano, 1998). Several factors may account for the lack of a stronger preference for tannins by wethers exposed to a high protein diet. Animals are more likely to learn about the benefits of a medicine when they experience malaise and subsequently relief contingent with the ingestion of the medicinal feed (Villalba et al., 2006a). In other words, a medicine needs to be ingested "at the right time" in order for an animal to learn about its benefits. Negative internal states such as toxicosis induced by tannins or acidocis promoted by high-grain diets are rapidly attenuated upon ingestion of neutralizing substances such as polyethylene glycol or sodium bicarbonate, respectively (Provenza et al., 2000; Phy and Provenza, 1998). In contrast, tannins do not neutralize the negative effects of excess N but attenuates the formation of ammonia in the rumen (Min et al., 2003). Thus, animals in the present study did not associate an ingestive event (i.e., tannin consumption) with a contingent reduction of a state of malaise (excess N), as malaise was likely prevented or attenuated by a reduced formation of NH3 in the rumen. Wethers likely associated tannin consumption with an improved physiological condition relative to previous days, when tannins were not offered as a supplement. Delayed associations between behavior (e.g., tannin ingestion) and its consequences (e.g., malaise alleviation) typically reduce the strength of the behavioral response (Mazur, 1994).

In addition to an appropriate timing for medicine ingestion, self-medicative behaviors may not always be expressed as animals may accept a certain amount of change or deviation in their internal state without modifying their feeding behavior. It has been suggested that an appropriate question to ask in terms of diet selection is "how much change or deviation in the internal state is the animal prepared to accept?" (Kyriazakis et al., 1999). Differences in animal species, physiology, tolerance and susceptibility may change the answer to that question and thus affect the outcome of learned self-medicative behaviors. Failure to select an appropriate diet or supplement may also depend on the conditions particular to a specific experiment (Tolkamp et al., 1998).

Despite the positive attributes of tannins at enhancing protein utilization, these compounds are better known for their anti-nutritional and toxic properties (Barry and McNabb, 1999). Quebracho tannins, particularly, have high affinity for proteins causing negative postingestive effects (Dawson et al., 1999). Thus, the potential benefits associated with consuming tannins (i.e., benefits) must be traded-off against their potentially negative effects (i.e., costs) (Hutchings et al., 2003). According to trade-off theory (Hutchings et al., 2006), in order for sheep to improve their physiological status from the nitrogen-binding properties of tannins, they should obtain a net benefit from tannin ingestion. This benefit may occur if the positive effects of tannins outweigh their anti-nutritional (negative) consequences on animal tissues and metabolic processes. The resultant from these opposing forces will obviously depend on their specific intensity on the herbivore's body. If the N-binding activity of tannins is more effective than their anti-nutritional activity, then sheep should have obtained a net benefit from tannin consumption and thus should have increased preference for tannin-containing foods. In contrast, if the N-binding activity of tannins was less intense than their anti-nutritional and toxicological activity, then sheep are not expected to obtain a net benefit, but a net cost from tannin consumption (Hutchings et al., 2006). In this case, a reduced preference is expected. In cases where the beneficial and negative activities of tannins have similar intensities, herbivores are not be expected to attain a net effect on their fitness/performance, as positive and negative effects will offset each other (Hutchings et al., 2006). Collectively, from the aforementioned analysis and the results obtained in the present study it is suggested that the costs of tannin ingestion were greater than its benefits, except when tests showed a tendency for a greater preference for tannins.

In addition to the toxic effects of quebracho tannins, the negative – and potentially toxic – impacts of the basal diet may have affected supplement preferences. Ingestion of diets high in ruminally degradable nitrogen lead to high concentrations of ammonia in the rumen which can escape from the liver and pass into peripheral circulation where toxicity may occur (Chalupa et al., 1970). Ammonia toxicity is mediated by various mechanisms in the brain (Felipo et al., 1993) and this is probably why sheep regulate food intake to maintain blood ammonia nitrogen levels below 2 mg/L (Nicholson et al., 1992). It is likely that in the present study excess ammonia was metabolized in the liver and thus did not reach levels that induced malaise. In these conditions, and according to trade-off theory (Hutchings et al., 2006), the net benefit of consuming tannins would be outweighed by the negative postingestive effects of tannins. This may explain the low preferences for the tannin-containing supplements observed in this study. Species and breeds with a greater efficiency at recycling nitrogen like Bedouin goats (Silanikove, 1997) may be more sensitive to excess ammonia and thus benefit to a greater extent from a tannin-containing supplement. Alternatively, the negative effects of excess ammonia may have interacted with the negative effects of tannins to attenuate supplement preferences. When sheep ingest two foods in the same meal they can attribute the postingestive effects of one food to the other food due to the close temporal proximity between both ingestive events (Yearsley et al., 2006; Villalba et al., 2006b; Freidin et al., 2011). Thus, it is possible sheep attributed some of the negative postingestive effects of the basal ration to the tannin-containing supplement which may be another reason preference for the tannin-containing supplement was low.

5. Conclusions

Our results suggest tannin supplements have the potential to reduce rumen ammonia nitrogen and BUN in sheep, even when fed after ingestion of high-N diets and in the form of low-quality supplements. When offered a choice between a tannin-containing supplement and the same supplement without tannins, wethers fed a high protein diet showed a trend for an increased preference for tannins relative to animals fed a diet of lower protein content.

Tannin supplementation may represent a viable alternative for maintaining high levels of nutrient intake in high-nitrogen rations or for enhancing N use in animals grazing pastures with high concentrations of N.

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