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Diet mixing and condensed tannins help explain foraging preferences by Creole goats facing the physical and chemical diversity of native woody plants in the central Monte desert (Argentina)

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ABSTRACT

The aim of this study was to understand the benefit of diet mixing for Creole goats grazing native forage species in the central Monte desert of Argentina and the drivers of preference in the mixed diet. To achieve this goal, cafeteria-style experiments were conducted with thirty female Creole goats (2 years old; 44 ± 1.4 kg) and forage species that are typically ingested by goats in this region: Two tanniniferous (*Tricomaria usillo*, *Mimosa ephedroides*) and three non-tanniniferous shrubs (*Prosopis flexuosa*, *Capparis atamisquea*, *Atriplex lampa*). In Experiment 1, goats were assigned to three groups ($n = 10$); two groups were offered single tannin-containing shrubs as their basal diet (*T. usillo*; SDTU or *M. ephedroides*, SDME), whereas the third group received a combination of all five forages (Mixed diet of forages, MD). After a fifteen-day adaptation period, half the animals in each group ($n = 5$) were dosed with polyethylene glycol (PEG), a polymer that neutralizes the negative effects of tannins, whereas the other half (Control) were not dosed (CG). Daily dry matter intake (DMI), *in vivo* apparent digestibility of the diet (ADD) and nitrogen (ADN) were determined. For goats fed MD, preference was estimated based on the DMI of each of the forages offered. Jugular blood samples were collected on the first and last days of the experiment to determine concentrations of blood urea nitrogen (BUN) and serum metabolites indicative of liver damage. In experiment 2, intake rates (IR) of the five forage species were estimated. No significant differences in DMI were detected among treatments. However, goats offered a choice of forages (MD) in the CG treatment had greater diet digestibility and lower BUN than animals fed the single shrubs, showing evidence of a nutritional benefit with dietary diversity. Goats changed their foraging preferences in response to PEG supplementation. Animals in the CG treatment preferred *M. ephedroides* whereas animals in the PEGG treatment preferred *A. lampa*. There was a positive correlation between forage preference and IR of crude protein ($r = 0.65$; $P < 0.001$) in PEGG goats, and between forage preference and IR of total tannins ($r = 0.77$, $P < 0.001$) in CG goats. When PEG attenuated biological effects of condensed tannins, goats switched their preference from forages that offered the greatest IR of total tannins (i.e., *M. ephedroides*) to those that led to the greatest IR of crude protein (i.e., *A. lampa*). In summary, a mixed diet led to greater nutritional benefits than

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single diets, which contribute to explain the diverse array of food items goats typically show when browsing in the central Monte desert of Argentina. Our results also show that CP, tannins and plant structure (which offer variable intake rates) play significant roles in goats' foraging preferences in this environment.

1. Introduction

Free-range grazing herbivores face the nutritional challenge of maintaining the constancy of their internal environment (i.e., homeostasis) despite the functional heterogeneity of the forage resources offered by the grazing environments. Among domestic ruminants, goats (*Capra hircus*) stand out for their wide distribution and better productive performance in arid and semi-arid rangelands. This has been explained mostly in terms of behavioral and physiological strategies distinctive of goats, which are continually shaped by the interplay between the environment and the genome (Provenza, 2008; Silanikove, 2000). As a result of the interaction between these forces, grazing goats select a balanced diet from many species that differ in their morphological and chemical characteristics (e.g., physical and chemical defenses, nutritional characteristics). This leads to the selection of three to five food items, which typically make up the bulk of a meal (Papachristou et al., 2005; Provenza et al., 2007). Likewise, in a desert region – the central Monte of Argentina – grazing Creole goats incorporate over fifteen forage species into their diet, out of which only five represent almost 70% of the diet (Allegretti et al., 2012; Egea et al., 2014). Among these species, the shrubs *Tricomaria usillo* and *Mimosa ephedroides* stand out, not only for their significant contribution to the bulk of the diet but also for their high content of phenolic secondary compounds (Allegretti et al., 2012; Egea et al., 2014).

Originally, plant secondary compounds (PSCs) were considered as by-products of plant metabolism and means for deposition of excesses of C fixed by photosynthesis. Although it is still unknown whether these compounds are involved in the primary metabolism of plants, there is no doubt about their involvement in defense against herbivory (Iason, 2005). Plant secondary compounds have been widely studied for their aversive orosensory and toxic effects, which have a deterrent effect on herbivores. However, more recent studies indicate that the intake of adequate amounts of some of these compounds can have positive effects on nutrition, productive performance, and even on the health of domestic ruminants (Waghorn, 2008). Among phenolic secondary compounds, condensed tannins constitute a good example of this duality, since they can be either harmful or beneficial to the animals (Makkar, 2010). The biological effects of tannins in animals does not only depend on the concentration of these components in the plants' tissues, but also on their chemical structure, interaction with other PSCs and/or nutrients in the diet, plant growth stage, specie and physiological condition of the animal, among other factors (Makkar, 2003; Min et al., 2003). The dissuasive power of tannins would be mediated by their deleterious effects on palatability, intake and digestibility of forage species (Goel et al., 2005). However, tannins are not completely avoided by goats, but rather are tolerated under certain threshold (Jansen et al., 2007; Egea et al., 2014). This may be related to benefits provided by intake of small amounts of tannins. For example, 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 enhances immune responses (Niezen et al., 2002) 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). Contrariwise, in the absence of tannins or when animals are dosed with substances that inactivate tannins, such as Polyethylene glycol (PEG), ruminal microorganisms could reach their maximum protein degradation rate with the consequent production of ammonia (NH3). Ammonia concentration may exceed the capacity of rumen bacteria to incorporate it in the synthesis of bacterial protein. The ammonia not incorporated in the synthesis of bacterial protein is absorbed through the rumen wall to be used as a precursor in the hepatic urea synthesis. Urea is transported by blood to the rumen and/or excreted in urine. As a result of the urea entering into the bloodstream, the concentration of blood urea nitrogen (BUN) increases (McMahon et al., 2000).

In addition to chemical defenses, some morphological traits of plants, such as specific leaf area (SLA), specific stem density (SSD), tensile strength and spines affect and/or restrict the food ingestion-digestion process and, consequently affect the feeding behavior of herbivores (Cooper and Owen-Smith, 1986; Sebata and Ndlovu, 2010). Studies realized by Egea et al. (2014) in the study area – the desert central Monte of Argentina – showed that free-range grazing Creole goats mainly consumed the terminal ends of growing shoots (stems with leaves and/or leafless) of woody species which were markedly different in their physical traits. In the same work, food selection of Creole goats could be better explained in terms of the mechanical features of food (SLA and SSD) than in terms of chemical features. Considering that herbivores crop between 10,000 and 40,000 bites per day from different individual plants (Illius et al., 1999), dietary decisions performed by goats based on the physical characteristics of forage have important implications for nutrient and PSCs intake rate and animal performance (Illius et al., 1999; Shipley et al., 1999).

From the perspective of the nutritional challenge that physico-chemical diversity of forage resources in arid and semi-arid ecosystems offer to goats, intake of a varied diet could be viewed as a behavioral strategy. This strategy would allow goats to achieve their nutritional requirements, counteract the deleterious effects of PSCs and, in some instances, benefit from the intake of adequate amounts of PSCs such as tannins (Villalba and Provenza, 2009). Previous results obtained in the study area provide clear evidence with respect to which parts and species of native plants are selected by free-range grazing Creole goats (Allegretti et al., 2012; Egea et al., 2014) and how chemical and morphological traits influence diet selection. However, the available information about goats' food preference and the mechanisms driving these preferences in such environments is still scarce. Therefore, the objective of this study was to better understand the benefit of diet mixing for Creole goats grazing native forage species that differ in chemistry and structure, as well as the drivers of preference in the mixed diet. The hypotheses tested in this study and their predictions are listed below:

(1) Tannins, like other plant bioactive substances, produce different biological effects in herbivores (pre- and post-ingestive consequences). These effects are closely related to tannin concentration and chemical structure in the forage species (Haslam, 2007). Based on this hypothesis, we expect to find differences in daily food intake and in *in vivo* apparent diet digestibility in Creole goats feeding from different tanniniferous shrubs.
(2) A diversity of food items (i.e., when the animals have the option to choose among alternatives), may improve food intake and/or nutrient utilization while reducing the negative impacts of PSC. This is because a diverse diet may increase the likelihood of nutrient–nutrient, PSC–nutrient and PSC–PSC interactions which benefit the consumer (Provenza et al., 2007). According to this hypothesis, we expected that the Creole goats fed mixed vs. single diets of tanniniferous and non-tanniniferous forages would show an improvement in daily food intake, and *in vivo* forage digestibility.
(3) Finally, plant morphology and plant chemical characteristics affect the rate of forage intake (Provenza, 1995; Wright and Vincent, 1996), which results in different post-ingestive consequences that can modify the animal's nutritional status and ensuing food preferences (Provenza, 1995). Based on this hypothesis, we predicted that goats would prefer shrubs that offer high rates of food intake but that such preference would be modulated by the chemical composition of the shrub.

We conducted controlled pen trials to explore the effects of forage diversity, condensed tannins and plant morphology on Creole goats' foraging behavior.

2. Materials and methods

2.1. Study area, animals and plants

This study was conducted from February to March 2014 in the Argentine Institute of Arid Zone Research (IADIZA), National Council of Scientific and Technical Research (CONICET), Mendoza province, Argentina. All experimental procedures and animal care practices were in agreement with the provisions of the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Trials were performed on adult Creole goats selected from a commercial meat goat flock in the household "La Majada", situated in the northeast (NE) of Lavalle (Mendoza) in the central Monte desert of Argentina (32°19′39′S, 67°54′36′W). Goat husbandry under extensive production is the main economic activity in this area (Guevara et al., 2006). The climate is arid and markedly seasonal, with cold dry winters (dry season) and hot wet summers (wet season). Shrublands and open woodlands play an important role because they provide forage for grazing animals throughout the year.

The vegetation communities of greatest forage importance are semi-closed woodlands of *Prosopis flexuosa* DC with *Atriplex lampa* Gillies ex Moq. in interdune valleys, and open woodlands of *P. flexuosa* with *A. lampa* and *T. usillo* Hook. & Arn. on dunes (Alvarez et al., 2006). The woody species most commonly found include the trees *P. flexuosa* and *Geoffroea decorticans* (Gillies. ex Hook. &Arn.) Burkart, and the shrubs *Bulnesia retama* (Gillies ex Hook. & Arn.) Burkart, *Capparis atamisquea* Kuntze, *A. lampa*, *T. usillo* and *M. ephedroides* Benth. Five of the major woody species consumed by grazing Creole goats during wet season in the NE of Lavalle (Mendoza province) (Egea et al., 2014; Allegretti et al., 2012) were utilized in the present study: *T. usillo*, *M. ephedroides*, *A. lampa*, *C. atamisquea* and *P. flexuosa*. By convention, based on condensed tannin (CT) concentration (% TC on dry matter basis) in these plants (Egea et al., 2014), two forage species were considered as tanniniferous plants (≥3% CT): *T. usillo* and *M. ephedroides*, and three species as non-tanniniferous plants (<3% CT): *P. flexuosa, C. atamisquea* and *A. lampa*. Plant nomenclature is according to Ruiz Leal (1972).

T. usillo is winter-deciduous shrub, 1–2 m tall, with an intricate branching pattern, stiff branches and densely pubescent leaves. *M. ephedroides* is an erect, ramose, almost leafless shrub, 0.50–1 m tall, with photosynthetic stems. *A. lampa* is an evergreen shrub, 0.5–1.5 m tall, densely branched and leafy, with somewhat fleshy leaves. *C. atamisquea* is a winter-deciduous shrub, 2–3 m tall, stiff branches, alternate and simple leaves densely covered with hairs only on lower. *P. flexuosa* is a winter-deciduous tree, 2–8 m tall, with somewhat pendulous and arching branches, geminate thorns and bipinnate leaves. Further details about morphological traits of forage species can be found in Ruiz Leal (1972) and Egea et al. (2014).

2.2. Experiment 1

2.2.1. Housing and general protocol

Thirty adult female Creole goats [2-years-old; 44 ± 1.4 kg (average ± SEM) live weight, LW] were placed in individual pens (1.5 m × 2 m), where they had free access to fresh water and trace mineral salt blocks. Goats were assigned to three groups

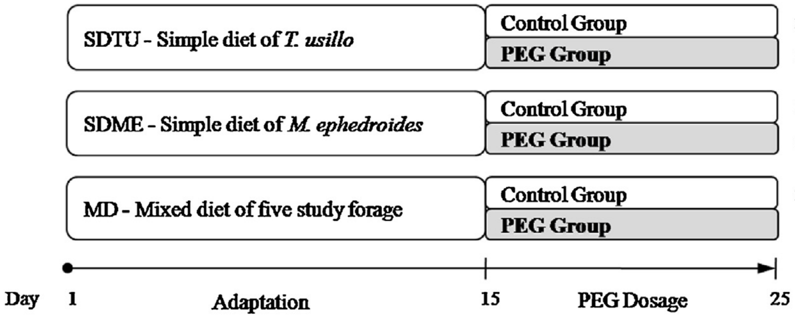

Fig. 1. Design of the Experiment 1.

($n = 10$) balanced for body weight and body condition score. Each group received a different diet comprising unbrowsed branches of tanniniferous plants, as the only forage in the diet (Single diets) and in combination with unbrowsed branches of non tanniniferous plants (Mixed diet). Single diets were branches of *T. usillo* (SDTU); or *M. ephedroides* (SDME) and the mixed diet was formed by offering a choice of *T. usillo, M. ephedroides, P. flexuosa, C. atamisquea* and *A. lampa* (MD). Branches of all forages offered consisted of current year's growth (approx. 70 cm long and 4 mm diameter) and were harvested four times a week from ungrazed plants at the grazing area of household “La Majada”. Harvested branches were placed in burlap bags and stored at 4 °C until used. This handling protocol prevents significant alterations of tannin profiles in forage species. In all three-treatment diets, forage species were offered *ad libitum* from 8:00 to 18:00 daily, and branches were mounted vertically at a 45° angle on artificial supports. All goats were supplemented with 200 g per day of a commercial pellet formulated for the specie and category animal. Pellets were formulated with corn, soybean meal, wheat bran, salt and mineral-vitamin premix to contain, on a dry matter basis, 5% moisture, 14% crude protein, 3% fat and 8% fibre. No other feed was offered until the next day. Goats remained within the same diet treatment throughout the study (25 days). All goats were weighed on days 1, 15 and 25, and were dewormed with ivermectin before the test.

After a fifteen-day adaptation period to become familiar with the individual pens, feeding and other housing management aspects, goats receiving the same diet (i.e., SDTU, SDME and MD) were assigned to two homogeneous groups ($n = 5$) [Control group (CG) and Polyethylene glycol group (PEGG)]; thereby, six groups of animals were defined as shown in Fig. 1. Each goat in PEGG was dosed orally with 50 g of polyethylene glycol [PEG (MW 4000), previously dissolved in water (1:1)] at 8:00 once daily for ten days; goats in CG were not dosed. Polyethylene glycol is an inert and unabsorbed molecule that can form a stable complex with tannins preventing binding between tannins and proteins and, therefore, inhibiting the effects of tannins on the animal (Waghorn, 2008). Its use has been a valuable tool to study pos-ingestive effects (i.e., intake and digestibility) of tannins in goats consuming tanniniferous plants (Silanikove et al., 1996; Villalba et al., 2002).

2.2.2. *Intake, digestibility and preference*

Fresh matter intake and total faecal output were measured individually and daily in all goat groups during the 10-day period of PEG dosage. Fresh matter intake was calculated as the difference between amount of food offered and refused, corrected for evaporative weight losses. To estimate weight loss due to transpiration, unbrowsed control branches of five forage species were weighted before and after the trials daily. Samples of offered and refused feeds were collected daily to determine dry matter (DM) and nitrogen (N) contents. Total faecal output was estimated by collecting faeces in bags attached to a harness fitted to each goat according to recommendations of Yiakoulaki and Nastis (1998) and Givens et al. (2000). Recommendations aimed at seeking the animal comfort, the accuracy of faecal output estimation and the minimizing of contact between urine and faeces. Bags were emptied at least two times a day or more often if necessary (determined by the volume of fresh faeces produced), the faeces taken each day were weighted and faecal subsamples were taken daily to determinate DM and N contents. These subsamples were bulked across days to generate one sample per goat.

Intake and faecal output of DM and N were calculated as the product of DM and N contents in diet components and faecal samples, and fresh matter intake and total faecal output, respectively. The *in vivo* apparent digestibility of diet (ADD) and nitrogen (ADN) were then calculated according to the following equations (Eqs. (1) and (2), respectively) (Bondi, 1989):

$$\mathrm{ADD} = \left(\frac{I_{\mathrm{DM}} - \mathrm{FO}_{\mathrm{DM}}}{I_{\mathrm{DM}}}\right) \times 100 \tag{1}$$

$$\mathrm{ADN} = \left(\frac{I_{N} - \mathrm{FO}_{N}}{I_{N}}\right) \times 100 \tag{2}$$

where I is the DM and N intake and FO is the faecal output of DM and N.

Preferences for the five woody species were assessed based on DMI of each forage species from groups fed the mixed diet (Alonso-Díaz et al., 2007). Since all five forages were offered at the same time, goats reached each branch by a lateral movement of the head without moving the whole body. The goats were offered all five woody species daily changing the order each day to avoid any bias in selection based on site preferences (Morand-Fehr, 2005).

2.2.3. Clinical biochemistry

Jugular blood samples were drawn from all goats on days 1 (baseline) and 25 to determine serum concentrations of blood urea nitrogen (BUN) and the serum enzymes aspartate aminotransferase (AST) and gamma glutamyltranspeptidase (GGT), following the methodology described by Silanikove and Tiomkin (1992). Blood urea nitrogen and serum enzymes concentrations have been conventionally used as indicators of pos-ingestive consequences of tannins in dietary protein utilization (Fernández et al., 2012) and hepatotoxic effects of tannins (Murdiati et al., 1990), respectively.

2.3. Experiment 2: Intake rates of nutrient and tannins

The day after completing the experiment 1, five goats from Experiment 1 were kept in individual pens. Goats had free access to fresh water and trace mineral salt block and were fed *ad libitum* amounts of alfalfa hay as the basal diet during 30 days. Bite size, bite rate and intake rates of DM, crude protein (CP), neutral detergent fiber (NDF) and total tannins (TT) were assessed for each one of the five plants used in the study. Before feeding basal diet, cut branches of each species (1.5 kg) were offered individually to each goat by mounting branches vertically at a 45° angle on artificial supports (the same supports that were used in experiment 1). Each of the five forage species was considered as a treatment. Each treatment was offered to each experimental goats ($n = 5$) during five consecutive days. In between treatments, animals were fed a basal diet of alfalfa hay for 1 day. During 40 min or until 75% of the offered branches were removed, number of bite and browsing time (time spent feeding) were recorded and refusals were weighed. The number of bites counted during feeding and the time spent feeding were used to calculate bite rates (bites min^{-1}), while bite sizes (g DM bite^{-1}) were calculated from the difference between initial and residual branch mass (after correcting for transpiration losses as described before) divided by the number of bites. The contents of CP, NDF and TT in bites (g CP, NDF and TT bite^{-1}) were estimated based on chemical composition and bite size of each one of five forage specie. The intake rates of DM (g DM min^{-1}) were estimated as the product of bite rate and bite size (Allden and Whittaker, 1970; Ungar, 1996). Intake rates of CP, NDF and TT (g CP, NDF and TT min^{-1}) were calculated as the product of contents of CP, NDF and TT in bites of each forage specie and their corresponding bite rates.

2.4. Chemical analysis

In experiment 1, daily samples of individual faeces and offered and refusal feeds (forage species and commercial pellet) were collected during the 10-day period of PEG dosage. In experiment 2, daily samples of forage species were collected during the 5-day recording period of each forage specie. All samples were placed in paper bags and dried in a forced-air oven at 60 °C for 48 h. They were subsequently ground through a Wiley mill with a 1-mm screen and analyzed for DM, NDF, acid detergent fiber (ADF), nitrogen (N) and CP contents. DM, NDF and ADF were determined by the procedure described by Goering and Van Soest (1970). Kjeldahl nitrogen was determined by the methods of AOAC (1990). Crude protein was calculated as 6.25 × N. Total phenols (TP), total tannins (TT) and condensed tannins (CT) were determined by spectrophotometry methods according to Makkar (2010). The Acid-butanol proanthocyanidin assay was used to determine CT, the Folin-Ciocalteu method was used to determine TP. Total tannins were obtained by the difference of TP before and after treatment with polyvinylpolypyrrolidone. Biological activity of tannins was assessed using the radial diffusion method (Hagerman, 1987). Tannin diffusion through an agarose gel containing bovine serum albumin (BSA) precipitate this protein forming a ring precipitate; the ring diameter is considered proportional to the ability of tannins to precipitate proteins, and therefore, to the astringency of these compounds (Mendes et al., 2006).

2.5. Statistical analyses

Analyses were computed using the GLM procedure of InfoStat statistical software (InfoStat, 2012). The covariance structure for each variable analyzed was chosen by comparing several models with different covariance structures. The variance-covariance structure yielding the lowest AIC value (Akaike's Information Criterion) was selected. The model diagnostics included testing for a normal distribution of the error residuals and homogeneity of variance. Differences between means were determined by the Fisher's LSD test. For transformed data, back transformed means are reported in the results. For all variables analyzed, significance was declared at $P < 0.05$ and tendencies at $P \leq 0.10$.

Dry matter, nutrients and tannin intake [grams ingested/kg metabolic body weight (BW $\text{kg}^{0.75}$)] were analyzed as a mixed model with repeated measures (day). Apparent digestibility of diet and nitrogen were analyzed as a two-way model of analysis of variance. Polyethylene glycol dosage and treatment diets were fixed effects, and individual animal was treated as random effect. Goats' preferences for forage species were assessed across last five days of experiment 1 (in goats fed mixed diets) and were analyzed as a split-plot design. The response variable was DMI of each forage specie as percentage of total DMI [(DMI each forage specie/DMI of five forage species) × 100)] (Heady, 1964). Group (PEG and control) was the between-animal factor, and animals were nested within group. Forage specie was the within-animal factor in the analysis and day was the repeated measure. Plant chemical characteristics and intake rates (DM, nutrients and tannins) of five study forages were analyzed using one-way ANOVA with forage species as fixed effect. Pearson correlation analyzes were completed between preference (DMI) and intake rates of forage species.

3. Results

3.1. Intake and digestibility

Daily total dry matter intake (DMI, g DM kg^{-1} BW$^{0.75}$) and apparent diet digestibility of diet and nitrogen (expressed as percentage of dry matter) are presented in Table 1. DMI was greater for goats fed a mixed diet of five studied forages (MD) and a single diet of *M. ephedroides* (SDME) than for goats fed a single diet of *T. usillo* (SDTU) (Table 1). Between goat groups feeding single diets of tanniniferous shrubs, DMI was greater ($P < 0.05$) for goat fed a SDME than for goats fed a SDTU (58.5 and 44.3 g DM kg^{-1} BW$^{0.75}$, respectively). There were non-significant differences in DMI between goats dosed with PEG or not. Moreover, goats of control group (CG) fed a mixed diet showed the greatest ($P < 0.05$) apparent diet digestibility (ADD; 64%), whereas for the remaining treatments, digestibility ranged from 37% to 48%. Control group goats which received the single diet of *M. ephedroides* reached the greatest ($P < 0.05$) apparent nitrogen digestibility (AND; 85%) while the lowest value (69%) was observed for CG goats fed single diet of *T. usillo* (Table 1).

Goats in all treatments reached values of daily nutrient intake according to their nutritional requirements (NRC, 2007). The higher protein intake in animals fed the mixed diet and dosed with PEG was attributed to the Diet ($P < 0.001$) and Dosing ($P < 0.05$) effects, respectively (Table 1). There were non-significant differences in consumption of NDF among groups of goats. Regarding the daily intake of TP, TT and CT, all animals consumed lower amounts of these compounds or very close to the limits considered harmful to ruminants (Waghorn, 2008; Makkar, 2010). Differences in daily intake of TP and TT were attributed to the diet effect ($P < 0.05$) (Table 1). TP consumption (% DMI) was higher ($P < 0.05$) in the groups of goats fed SDTU and SDME (5.1% and 5.8%, respectively) than in groups of goats fed MD (3.1%), whereas in decreasing order (i.e. from high to low consumption), TT consumption was higher in goats fed SDME (2.4%), next, in those receiving SDTU (1.8%), and finally, in goats fed MD (1.3%). Goats fed SDME and MD increased CT consumption when they were dosed with PEG. The same was not observed in goats fed STDU, as PEG dosage did not increase CT ingestion. Goats of PEGG fed the MD and the SDME showed the greatest ($P < 0.05$) CT consumptions (Table 1).

Among the goats fed the mixed diet, there were significant differences ($P < 0.05$) in dietary preferences between goats of CG and PEGG. Among goats of CG, the most preferred species in decreasing order were *M. ephedroides, T. usillo* and *P. flexuosa* (with non-significant differences in consumption), *A. Lampa* and *C. atamisquea*. Among goats of PEGG, *A. lampa* was the most preferred forage followed by *P. flexuosa*, *T. usillo* and *M. ephedroides* and, as in the CG, *C. atamisquea* was the least preferred (Fig. 2).

Table 1
Dry matter, nutrient and tannin intake (g kg^{-1} BW$^{0.75}$), and apparent diet digestibility of diet and nitrogen (% DM) in goats dosed with or without PEG (Control and PEG groups, respectively) fed single diets of tanniniferous shrubs and mixed diet of five forage species tested.

Diets		Daily intake						Digestibility	
		DM	CP	NDF	TP	TT	CT	Diet	Nitrogen
Control group									
SDTU		41.6	5.8	17.3	2.6	0.8	0.2^{AB}	47.5^{A}	69.4^{C}
SDME		51.7	6.8	18.3	2.6	1.2	0.3^{A}	45.4^{A}	84.6^{A}
MD		56.7	8.7	21.3	2.0	0.9	0.2^{B}	**64.5^{B}**	74.8^{B}
PEG group									
SDTU		46.3	5.6	16.1	2.5	0.8	0.2^{AB}	37.4^{A}	76.7^{B}
SDME		64.1	7.7	24.7	3.5	1.7	0.4^{C}	45.7^{A}	75.6^{B}
MD		61.9	10.7	22.5	2.0	0.8	0.4^{C}	47.5^{A}	76.3^{B}
Overall mean									
Control group		57.6	7.1*	19.1	2.4	1.0	0.21*	52.5*	76.3
PEG group		51.2	8.0	20.1	2.7	1.1	0.31	43.5	76.2
SDTU		44.3^{a}	5.7^{a}	16.7	2.5^{ab}	0.8^{a}	0.21^{a}	42.5^{a}	73.1^{a}
SDME		58.5^{b}	7.3^{b}	21.5	3.0^{b}	1.5^{b}	0.30^{b}	45.6^{a}	80.8^{b}
MD		60.4^{b}	9.7^{c}	21.9	2.0^{a}	0.9^{a}	0.26^{ab}	**57.6^{b}**	75.4^{a}
Group	SEM	–	0.3	–	–	–	0.02	2.0	–
	P-value	ns	<0.10	ns	ns	ns	<0.001	<0.05	ns
Diet	SEM	3.9	0.4	–	0.3	0.1	0.01	1.5	0.8
	P-value	<0.05	<0.001	ns	<0.05	<0.05	<0.05	<0.001	<0.001
Group × diet	SEM	–	–	–	–	–	0.03	3.6	1.2
	P-value	ns	ns	ns	ns	ns	<0.05	<0.10	<0.001

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; TP, total phenols; TT, total tannins; CT, condensed tannins; SDTU, simple diet of *T. usillo*; SDME, simple diet of *M. ephedroides*; MD, mixed diet of five study plants; SEM, standard error of the means; ns, non significative ($P > 0.05$). Means with different lowercase letters ($^{a–c}$) in the same column indicate significant differences between diets ($P < 0.05$). Means with different capital letters ($^{A–B}$) in the same column indicate significant differences related to Group × Diet interaction ($P < 0.05$).

Means with (*) in the same column indicate significant differences between treatments (PEG and control) ($P < 0.05$).

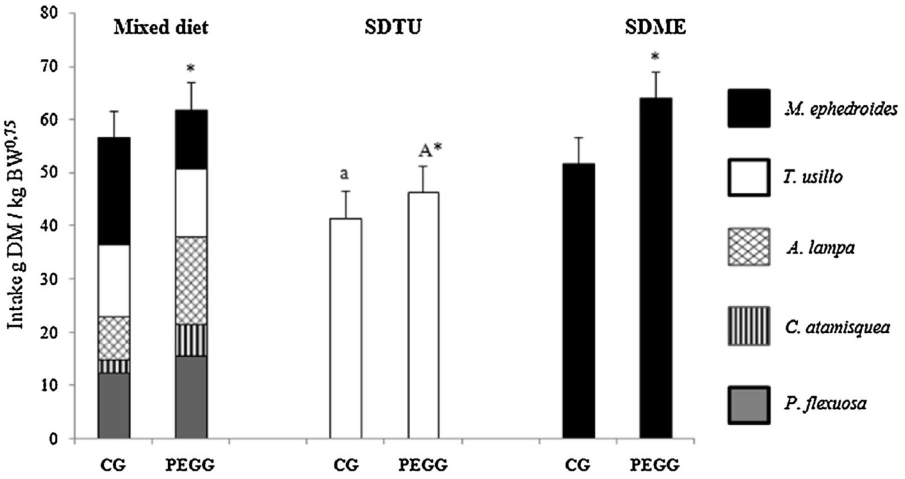

Fig. 2. Mean values of consumption (g DM kg^{-1} BW$^{0.75}$) in goats dosed and not dosed with PEG (PEGG and CG, respectively) fed single diets of tanniniferous plants (*T. usillo*, SDTU, or *M. ephedroides*, SDME) and a mixed diet of five forage species tested (Mixed diet) during the last five days of Experiment 1. Different lowercase letters indicate significant differences between diets in CG goats ($P < 0.05$). Different capital letters indicate significant differences between diets in PEGG goats ($P < 0.05$). (*) Indicate tendency between treatments (CG and PEGG) within a same diet ($P \leq 0.15$).

3.2. Clinical biochemistry

All the metabolites tested fell within the normal range for goats (Mitruka and Rawnsley, 1977) (Table 2). There were non-significant differences in blood urea nitrogen (BUN) concentration and serum enzymes between goats fed different diets. However, BUN was higher ($P < 0.001$) for goats dosed with PEG under mixed and single diets (Table 2).

3.3. Chemical analysis of forage species

Table 3 shows the chemical composition of forage species offered to goats in Experiment 1 and 2. CP content (as percentage of DM) in all test plants was in the range of 9 to 14% and it was greater ($P < 0.05$) in *C. atamisquea* and *P. flexuosa* (14 and 12%, respectively). Most test plants had NDF contents close to 55%, except for *A. lampa* which had the lowest NDF content ($P < 0.05$), nearly 50% less than other plant tested. Among tanniniferous shrubs, the high content and astringency of condensed tannin in *T. usillo* ($P < 0.05$) was highlighted.

3.4. Preference and correlations between preference and intake rates of nutrients and tannins

The mean values of bite size, bite rate and intake rates (IR) of nutrients and tannins estimated for the five forages studied are shown in Table 4. *A. lampa* was the forage species from which goats reached the highest values ($P < 0.001$) of bite size (1.4 g DM bite^{-1}) and intake rate of DM (12.1 g DM min^{-1}) and CP (1.2 g CP min^{-1}), whereas *P. flexuosa* and *T. usillo* were the forage species with greater ($P < 0.001$) intake rate of NDF (3.9 and 3.4 g NDF min^{-1}, respectively). Finally, from *M. ephedroides* and *T. usillo*, goats reached intake rates of TT (0.19 and 0.16 g TT min^{-1}, respectively) higher than from other forages ($P < 0.001$).

Both groups of goats (i.e., CG and PEGG) showed a positive correlation between preference and intake rate of NDF (CG: $r = 0.52$; PEGG: $r = 0.49$; $P < 0.001$). Intake rates of CP and TT show a positive relationship with forage preferences. A positive

Table 2
Blood metabolites of goats dosed with or without PEG (Control and PEG groups, respectively) fed single diets of tanniniferous shrubs and mixed diet of five forage species tested.

Blood metabolites	Mixed diet			Single diets					
				T. usillo			*M. ephedroides*		
	PEG	Control	*SEM*	PEG	Control	*SEM*	PEG	Control	*SEM*
BUN (mg dL^{-1})	0.41*	0.29	0.04	0,40*	0.27	0.03	0,43*	0.30	0.05
AST (IU L^{-1})	66.2	66.6	4.3	59.2	56.9	6.1	61.3	68.6	5.2
GGT (IU L^{-1})	10.6	10.4	2.5	11.06	12.0	1.3	17.7	13.9	2.0

Values are means ($n = 5$); SEM, standard error of the mean. BUN, blood urea nitrogen (mg dL^{-1}); AST, aspartate aminotransferase enzyme (IU L^{-1}); GGT, glutamyl transpeptidase enzyme (IU L^{-1}).
Means with (*) in the same row and within the same diet indicate significant differences between treatments (PEG and control) ($P < 0.05$).

Table 3
Chemical composition and tannin astringency of forage species tested. Values are means (SD).

Species	DM[*]	CP[*]	NDF[*]	ADF[*]	TP[†]	TT[†]	CT[††]	BARD[§]
Tanniniferous plants								
T. usillo	41 ± 3.2	9 ± 0.2	55 ± 0.5	37 ± 0.3	8 ± 0.6^{a}	2 ± 0.2^{a}	17 ± 0.2^{a}	5.6 ± 0.2^{a}
M. ephedroides	46 ± 2.8	10 ± 0.1	52 ± 0.2	39 ± 0.6	7 ± 0.5^{a}	3 ± 0.2^{a}	4 ± 0.2^{b}	4.9 ± 0.2^{b}
Non-tanniniferous plants								
A. lampa	30 ± 2.0^{a}	11 ± 0.7	27 ± 0.6^{a}	17 ± 0.6^{a}	na.	na.	–	–
C. atamisquea	58 ± 3.1	14 ± 0.9^{a}	51 ± 0.3	30 ± 0.8	1 ± 0.3	1 ± 0.1^{b}	2 ± 0.2	–
P. flexuosa	49 ± 2.8	12 ± 0.4^{a}	58 ± 0.3	47 ± 0.5	2 ± 0.2	1 ± 0.2	2 ± 0.2	–

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; TP, total phenols; TT, total tannins; CT, condensed tannins; BARD, biological activity measured as radial diffusion (tannin astringency); na., negligible amounts; –, not detected. Different letters in the same column indicate significant differences between forage species tested ($P < 0.05$).

[*] Expressed as percentage on a dry matter basis (%).

[†] Expressed as tannic acid equivalent on a dry matter basis (%).

[††] Expressed as leucocyanidin equivalent (g kg^{-1} DM).

[§] Measured as units of precipitation per g of plant relative to tannic acid.

Table 4
Bite sizes, bite rates and intake rates (IR) of nutrients and tannins estimated for the five forages studied. Values are means (SD).

Species	Bite sizes (g bite^{-1})	Bite rates (bite min^{-1})	Intake rates (g DM min^{-1})	Intake rates (g min^{-1})		
				CP	NDF	TT
A. lampa	1.4 (0.10)^{a}	8.9 (0.7)	12.1 (0.6)^{b}	1.2 (0.10)^{d}	3.0 (0.3)^{ab}	na.
C. atamisquea	0.4 (0.07)	7.4 (0.9)	2.8 (0.7)^{a}	0.3 (0.07)^{a}	1.5 (0.3)^{a}	na.
M. ephedroides	0.8 (0.03)^{b}	6.9 (0.4)	5.2 (0.4)	0.5 (0.02)^{ab}	2.6 (0.2)^{b}	0.19 (0.02)^{a}
P. flexuosa	0.7 (0.05)^{b}	9.1 (1.2)^{a}	6.5 (0.6)	0.8 (0.06)^{c}	3.9 (0.3)^{c}	0.06 (0.01)^{b}
T. usillo	0.4 (0.02)	14.9 (0.6)^{b}	6.1 (0.4)	0.6 (0.03)^{bc}	3.4 (0.2)^{bc}	0.16 (0.01)^{c}

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; TT, tannin total; na., negligible amounts. Different letters in the same column indicate significant differences ($P < 0.05$).

correlation between preference and intake rate of CP was observed in goats of PEGG ($r = 0.65$; $P < 0.001$), but not for goats of CG. When goats were not dosed with PEG, intake rate of TT was a good predictor of dietary preferences ($r = 0.77$, $P < 0.001$).

4. Discussion

As was propose in the first hypothesis, differences in biological effects of tannins could be explained in relation to concentration and chemical structure of these phenolic compounds in plants. Our results suggest differences in condensed tannins between the two tanniniferous forage species (*T. usillo* and *M. ephedroides*) studied. Goats fed SDME showed greater intake capacity than those fed SDTU, an outcome which could be explained by pre-digestive processes such as astringency, supported by the greater concentration and astringency of CT in *T. usillo* than in *M. ephedroides*. Increasing friction inside the mouth (i.e., astringency) is due to condensed tannins bind and precipitate salivary mucoproteins, which alter the lubricating properties of saliva (Glendinning, 2007). On the other hand, condensed tannins can negatively affect food intake not only due to their concentration (Wang et al., 1996a) but also due to their chemical structure and molecular weight and by the concentration of proteins in diet (Hagerman and Butler, 1981). Higher apparent digestibility of nitrogen (ADN) in goats fed SDME than goats fed SDTU (69% and 85%, respectively), as well as the significant increase of ADN in goats fed SDTU (69% to 77%) when were dosed with PEG, could also be explained in terms of concentration and chemical structure of tannins and affinity between these bioactive principles and dietary protein. The positive effect of PEG on nitrogen digestibility has been reported by other authors, who observed an increase in nitrogen digestibility in goats fed a tanniniferous forage (*Pistacia* sp.) when animals were dosed with PEG (Decandia et al., 2000).

The second hypothesis, about diet mixing by herbivores and the potential nutritional benefits due to complementarity among foods, was partially supported by the results obtained in this study. Even though there were no significant differences in dry matter intake between goats fed single and diverse diets, goats reached a greater apparent diet digestibility when were allowed to choose among an array of forage species. Greater diet digestibility in goats fed the mixed diet, together with lower concentration of blood urea nitrogen in goats of control group was interpreted from two points of view. On the one hand, (i) the post-ingestive effects of tannins, specifically in the ruminal digestion of protein, and on the other hand, (ii) the role of diet diversity as a behavioral strategy to optimize macronutrient and tannins intake. The first aspect (i) could be explained in terms of the inhibitory effects of tannins in proteins hydrolysis by ruminal bacteria (Tanner et al., 1994). As it was pointed out previously, in absence of tannins or when animals are dosed with substances that inactivate tannins (such as PEG) ruminal microorganisms could reach their maximum protein degradation rate with the consequent production of ammonia (NH3), which may exceed the capacity of rumen bacteria to incorporate it in the synthesis of bacterial protein. This mechanism allows explaining the higher blood urea nitrogen concentration in goats dosed with PEG than in goats not dosed with PEG. Similar results have been reported by Woodward (1988) who observed a decrease in blood urea nitrogen and rumen ammonia concentration in sheep and goats fed tannin-containing legumes. From the viewpoint of nutrients utilization, the decline in ruminal protein degradation associated to tannins, increases the amount of non-ammonia nitrogen reaching the small intestine (Waghorn et al., 1987). Studies carried out in goats (McAllister et al., 2005; Pérez-Maldonado and Norton, 1996) have demonstrated that intestinal pH facilitates separation of the tannin-protein complexes and subsequent intestinal absorption of amino acids. In the present study, there were no differences in nitrogen digestibility among PEGG and CG goats fed a mixed diet. This might be related to the typical increase in faecal excretion of nitrogen in ruminants consuming forages of low tannin concentration (Waghorn et al., 1994). Under such conditions, the "paradoxical" decrease in nitrogen digestibility may lead to misinterpretations if the benefits of switching the site of protein digestion (rumen versus intestine) are not considered (Waghorn et al., 1994).

Better use of dietary protein due to the mechanisms previously described has been linked to an increase in the productive performance of goats by Wang et al. (1996b). However, when forages have high tannin concentrations and goats have little or no option to select from different foods, productive performance may be adversely affected (Barry, 1985). This is closely related to the second aspect (ii) previously mentioned about the role of diet mixing as a behavioral strategy to optimize nutrient and tannin intake. Greater food intake and digestibility associated with greater dietary diversity has been reported in an increasing number of studies. These results have been explained in terms of an increased motivation to eat when animals have more than one type of food available (Ginane et al., 2002; Distel et al., 2007; Wang et al., 2010) and in terms of complementary intake of nutrients-PSCs (Rogosic et al., 2006; Villalba et al., 2010; Meier et al., 2014). Ingestion of single foods or flavors may reduce the motivation to eat (Rolls, 1986; Villalba et al., 2010) which leads to sensory-specific satiety. Ginane et al. (2002) concluded that in situations where animals can choose different food items, even when items of higher nutritional quality are preferred, lower quality foods help to increase motivation to eat for the mere fact of that they increase dietary diversity.

Finally, in according with predictions of third hypotheses, nutrient and tannins intake rates were different among the tested plants and, allow explaining at least in part the dietary preferences of goats. Both chemical and structural attributes of plants as well as the animal's physiological state influence rates of food intake (Villalba and Provenza, 1999). The positive correlation between fiber intake rate and preference for woody plant species has been reported by other authors (Dziba et al., 2003; Alonso-Díaz et al., 2007). Herbivores may require an optimum density of fiber in their food to access cellular contents, as fiber provides a rigid material against which cells are pressed until its rupture during chewing (Wright and Vincent, 1996). In addition, the fiber intake have been relates to higher volume of saliva buffer to achieve a greater ruminal pH in ruminants fed diets rich in tannins (Makkar, 2010). This favors ruminal fiber digestion and hence more energy available to the animal (Weimer, 1996).

Moreover, our results show a positive correlation between preference and intake rate of crude protein for goats dosed with PEG, but not for control goats. When effects of tannins were blocked by PEG (i.e. PEG group), goats increased consumption of the forage (*A. lampa*) which showed the highest intake rate of crude protein. Thus, the presence of tannins in the diet attenuated preferences for a forage that otherwise would be preferred due to its protein content, suggesting that goats tended to maximize consumption of protein when tannins were absent. In contrast, the intake rate of tannins was a good predictor of dietary preferences when goats were not dosed with PEG (i.e. Control group). In addition, these animals increased intake of *M. ephedroides*, forage specie that showed the highest intake rate of tannins. These suggest that control goats tended to maximize intake of this compounds, likely due to their benefits on nutrition and health (Waghorn, 2008; Provenza et al., 2003). Even when negative correlations between tannins and food choices by herbivores have been highlighted in numerous studies, tannins have also been widely recognized as good predictors of goat dietary preferences for different species of woody plants (Dziba et al., 2003; Alonso-Díaz et al., 2007). Many of these results have been interpreted from a physiological point of view, based on the post-ingestive effects of phenolic compounds. Furthermore, it is known that animals would be able to perceive the sensory clues of plants that provide them with immediate information about the nutritional properties of food, which has been regarded as one of the mechanisms underlying the food choices of herbivores (Edwards et al., 1997). In the present preference experiments, goats had previous browsing experience with all tested plants as these forage species were native to the region where experimental goats were raised. In this region, tanniniferous shrubs *M. ephedroides* and *T. usillo* are two of the main forage species selected by grazing goats (Allegretti et al., 2012; Egea et al., 2014). As was suggested by Villalba et al. (2015), foraging behavior is affected by the consumer's past experiences with the biochemical context in which a food is ingested, including the kinds and amounts of nutrients and plant secondary compounds in a plant and its neighbors. In addition, past experiences with food have the potential to influence food preference and intake through a mechanism, which is not entirely dependent on the classical homeostatic model of appetite control. Ruminants learn about the post-ingestive effects of nutrients (Provenza et al., 2003) and form preferences for forages that structurally offer high intake rates (Carvalho, 2013). Our results using tanniniferous forages and PEG show that protein, phenolic compounds and plant structure – through its impact on intake rates – play significant roles in goats' foraging preferences.

5. Conclusion

Our results provide some insights into Creole goats' intake of and preference for woody forage species. Greater digestibility in groups fed combinations of forages than in groups fed single shrubs suggest a nutritional benefit of dietary diversity. Blockade of tannins induced animals to increase preference for a nitrogen-rich shrub, which suggest that preference for this food item is constrained by tannins in natural environments. In contrast, preference for certain tanniniferous forages which offer high intake rates of TT is also possible in natural conditions which suggest that some tannins provide consumers with some positive effects (e.g., antiparasitic, antioxidant, improved protein nutrition). In conclusion, protein, tannins and structure all play significant roles in goats' foraging preferences in the central Monte desert, Argentina.

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