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A tannin-blocking agent does not modify the preference of sheep towards tannin-containing plants

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HIGHLIGHTS

- When given a choice of plants, sheep can ingest tannin-containing plants.
- Sheep preference is observed from the first hour of intake.
- Supplementation with a tannin blocking agent (PEG) does not modify preference.
- Tannins do not seem to regulate sheep preference or intake, as fiber content does.

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ABSTRACT

Sheep have been suggested to use their senses to perceive plant properties and associate their intake with consequences after ingestion. However, sheep with browsing experience do not seem to select against tannin-rich browsing materials in cafeteria trials. Thus, the objective of the present study was to evaluate the relationship between the chemical composition, selectivity index (SI), preference and intake rate (IR) of tannin-containing forage trees offered to sheep in cafeteria experiments. Four trees were selected for their condensed tannin content and their varying biological activities. *Havardia albicans* (high biological activity), *Leucaena leucocephala* (medium biological activity), *Acacia gaumeri* (low biological activity) and *Brosimum alicastrum* (very low biological activity) were used in this study. Ten hair sheep (23.7 kg ± 1.43 LW) with eight months of browsing experience in native vegetation were used in this study. Polyethylene glycol (PEG 3600 MW) was administered to five sheep during all experiments. In experiment 1, fresh foliage from all trees was offered ad libitum for 4 h. In experiment 2, *B. alicastrum* was withdrawn and the preference was determined again. The forage preference in experiment 1 was *A. gaumeri* (14.77 g DM/kg LW) > *B. alicastrum* (11.77 g DM/kg LW) > *H. albicans* (3.71 g DM/kg LW) = *L. leucocephala* (1.87 g DM/kg LW) ($P < 0.05$). The preference in experiment 2 was *A. gaumeri* > *H. albicans* = *L. leucocephala*. PEG administration had no effect on the preference or IR. The intake rate seemed to have been affected by the plant density. Moreover, fiber compounds were found to be better predictors of DM intake than polyphenolic compounds at levels typically found in the evaluated forages. It was concluded that tannins and PEG did not modify the preferences of sheep in cafeteria trials. Thus, tannins are not involved in the preference regulation of animals with browsing experience.

1. Introduction

Most tropical trees and shrubs have relatively high levels of plant secondary metabolites (PSM) [21,27]. Those PSMs, specifically condensed tannins (CT), have been reported as modulators of the intake of single feed under controlled conditions because large quantities of CTs seem to reduce intake (see review [16]). The role of condensed tannins in the feed preference during short term cafeteria experiments has been challenged in goats and sheep with browsing experience [1,3]. Both the fiber components and foliage density have been suggested to be involved in the regulation of IR [1,3]. Although some studies have found that adding a tannin blocking agent (polyethylene glycol, PEG) modifies shrub preference in goats and sheep [26,31]. A recent experiment with goats [14] showed that adding PEG to the tannin-rich browse offered in a cafeteria trial did not modify the animals' preferences. This result was also found by Rogosic et al. [26]. In addition, Favreau et al. [12] suggested that unless the sheep were conditioned, they preferred umami tastes and

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were indifferent to bitter tastes. The umami taste can be associated with protein content, while the bitter taste can be associated with tannin content. Hence, we hypothesized that the CT content of browse legumes was not a limiting factor in the feed preference of sheep and goats. Thus, adding the tannin-blocking agent PEG should not affect animal preference and selectivity. Therefore, the objective of the present study was to evaluate the relationship between the chemical composition, the selectivity index (SI), the preference and the intake rate (IR) of tannin-containing forage trees offered to sheep in cafeteria experiments.

2. Materials and methods

2.1. Study area

This work was carried out at the Faculty of Veterinary Medicine and Animal Science, Universidad Autonoma de Yucatan (FMVZ-UADY). The climate of the area is tropical and sub-humid, with summer rainfalls. The average temperature varies from 26 to 27.8 °C and the annual rainfall ranges from 940 to 1100 mm.

2.2. Experimental forages

The fresh leaves of the legumes *Havardia albicans* (HA), *Acacia gaumeri* (AG), and *Leucaena Leucocephala* (LL) have been reported as being rich in polyphenolic compounds [9,15]. *Brosimum alicastrum* (BA) has been reported as being preferred by cattle [28] and sheep [37]. Because BA has a low concentration of condensed tannins, this species was also included in the present study.

2.3. Experimental animals

Ten female Pelibuey hair sheep (initial average weight 23.7 kg ± 1.43, 10–11 months old) were used. All of the animals had eight months of browsing experience in the native deciduous tropical forest of the area. The animals were drenched with an effective wide spectrum anthelmintic three days prior to the first adaptation period.

2.4. Preference experiment 1

The animals were divided in two groups (n = 5): with and without addition of 25 g polyethylene glycol (PEG, MW 3600, Sigma Co.). PEG was diluted in water (1:1 w/v) and was dosed directly into the mouth before and 30 min after fresh leaves were offered [32].

The animals were allocated into individual pens (3 m × 3 m). Each animal was fed fresh grass (*Pennisetum purpureum*) ad libitum and 200 g of a grain-based concentrate (wheat bran, soybean meal and sorghum grain) every day. Fresh leaves (50 g) of each plant were offered together on a daily basis during a five-day adaptation period. The amount offered (50 g each plant) was selected to ensure their total ingestion and that the animals had previous knowledge of the plants offered (no refusals were observed). Then, the preferences of the animals were measured using a multiple Latin square design [6]. The animals were offered fresh leaves of each plant ad libitum in individual plastic feeders for 4 h periods. The positions of the feeders were changed daily to avoid conditioned learning (association) between the feeder positions and the forage species. Based on the preliminary observations of the container capacity, at least 200 g fresh leaves were always available. Refusal was measured every hour to obtain the cumulative intake and the containers were replenished with the measured amounts.

After the 4 h period, the tree forages were withdrawn and the animals received concentrate feed (200 g) and grass (ad libitum). The food was offered for 15 h only. The remaining 5 h animals were maintained without feed. Daily feed samples were collected, dried (60 °C), milled (1 mm sieve) and kept in airtight containers for later analysis.

2.5. Preference experiment 2

In this experiment, the choice of forage was restricted to the three tannin-containing plants (HA, AG, LL). This experiment was managed in a similar manner to experiment 1 but was preceded by a 4-day adaptation period. The feeding and sampling procedures were performed as described above.

The animals in both experiments adapted well to the experimental conditions. The pens were constructed with wire mesh, which allowed for visibility among the animals. The animals had apparently normal behavior and none of them were removed from the experiment due to abnormal behavior or disease.

2.6. Variables

2.6.1. Total foliage intake (TFI) and intake rate (IR)

TFI was recorded as the difference between the weight of the feed offered and the weight of the foliage remaining after the 4 h of consumption. For each animal, the time spent feeding (minutes) on each type of foliage during hours 1 and 4 was recorded. The IR was calculated as the amount of feed consumed (DM) per effective minute spent eating each type of feed. The average IR was calculated by pooling the data from hours 1 and 4.

2.6.2. Foliage density

To assess the effects of foliage structure on the DM intake, the foliage density of each plant was determined by placing 200 g of fresh foliage from each plant in containers. No attempt was made to press the foliage into the container. Then, the volume (cm^3) occupied by the foliage was measured. The density (w/v) was measured three times for each plant.

2.6.3. Selectivity index (SI)

The SI was calculated for each sheep daily during the experimental periods [10]:

For Experiment 1,

$$SI = \left[(1/4-P_i)\text{^}2 + \left(1/4-P_j\right)\text{^}2 + (1/4-P_k)\text{^}2 + (1/4-P_l)\text{^}2\right]/(3/4);$$

For Experiment 2,

$$SI = \left[(1/3-P_i)\text{^}2 + \left(1/3-P_j\right)\text{^}2 + (1/3-P_k)\text{^}2\right]/(2/3);$$

where P_i, P_j, P_k and P_l are the proportions of the food consumed per day. An animal was qualified as either completely selective when only one plant was consumed (index = 1) or as completely unselective when the same proportions of each plant species were consumed (index = 0)

2.7. Laboratory analysis

The samples were analyzed for their DM, N and ash contents (7.007, 2.057 and 7.009, respectively) according to A.O.A.C. [4]. NDF and ADF were uncorrected for residual ash, and NDF was determined using sodium sulfite without alpha amylase. The lignin content was also determined [33]. In addition, the cellulose (Cel) (calculated by the difference between ADF and Lignin), hemicellulose (Hem) (calculated by the difference between NDF and ADF) and cellulose + hemicellulose (Cel + Hem) contents were estimated. The foliage samples were oven-dried at 50 °C, ground and extracted using acetone:water (70:30 v/v). The samples were sonicated for 20 min and were then centrifuged for 10 min. The supernatants were used to determine the presence of phenolic compounds, including the total phenols (TP) (using gallic acid as standard) [24] and total tannins (TT) (Folin–Ciocalteu + PVPP method, [19]) using tannic acid as a standard. The condensed tannins (CT) were determined by butanol HCL (anthocyanidin equivalent, [23]) and the vanillin assay (catechin