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Effect of offering a multiple choice among Brazilian woody plants on intake and feeding behavior of experienced and inexperienced Santa Inês lambs



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ABSTRACT

The aim of the study was to investigate whether a diversity of woody feeds on offer, containing different phenolic amounts, and previous experience with the feeds have an effect on feed intake and feeding behavior of growing lambs. A choice feeding experiment was conducted in Northeastern Brazil (Teresina, Piauí) using chopped leaves and small twigs from five different woody plants (*Cenostigma macrophyllum*, *Combretum leprosum*, *Orbignya phalerata*, *Thiloo glaucocarpa*, *Trema micrantha*). Twelve weaned Santa Inês lambs were individually penned and either allocated to a treatment which allowed them to obtain experience with the test feeds or one where they remained inexperienced ('single feed test'). In five 7-day sub-periods (Test period 1), the 'experienced' animals received successively each of the five test plants alone during 6 h of test feeding in the morning. For the remaining 18 h the animals received a basal diet consisting of Bermuda grass (*Cynodon dactylon*) hay ad libitum and concentrate according to requirements. The 'inexperienced' animals were fed only with the basal diet during the 6 h of test feeding. Before starting the multiple choice tests, all animals underwent 10 days of feeding only the basal diet. The first multiple choice test (Test period 2) was designed to measure both, the effect of experience ('experience testing') and the effect of a diverse offer vs. hay as control ('multiple choice vs. hay'). Accordingly, at first three 'experienced' and three 'inexperienced' animals were offered a multiple choice among all woody plants on offer ('choice'), while the other three animals of the respective treatment received only hay ('control') during test feeding. After 10 days, the groups were switched, resulting in $n=6$ per treatment. The second 10-day multiple choice test (Test period 3; 'multiple choice vs. test plant') was designed to assess the effect of offering all woody plants as a multiple choice vs. only the woody plant most preferred in the previous tests (*T. micrantha*). Data were evaluated by various statistical models. *T. micrantha* was the most preferred plant among the five woody plants tested in terms of intake and feeding behavior, followed by *O. phalerata*. The other plants were mostly neglected, both when offered as single feed or in a multiple choice. Experience did overall not affect ($P > 0.05$) intake as tested in Test period 2 ('experience testing'). However, inexperienced lambs decreased total daily intake, including hay intake, as well as intake of *C. macrophyllum* and *O. phalerata* from the first to the last day of the test period, showing increased aversive behavior. Offering a multiple choice among different woody

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forages during 6 h/day ('choice') compared to only hay ('control') increased total daily forage intake from 27.3 to 32.1 g/kg body weight ($P < 0.001$). It was concluded that the 'control' group tried to diversify their diet when having a multiple choice option. However, when *T. micrantha* was offered as 'control' feed compared to a 'choice' group receiving all five test plants during 6 h test feeding, these differences disappeared. This might be mainly due to almost neglecting all other plants on offer apart from *T. micrantha* in the 'choice' group, leading to a similar diet of both groups.

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1. Introduction

When certain forage sources like grasses and herbs are getting dry in the semi-arid tropics for seasonal reasons, small ruminants on rangelands either have to eat the dry plants or incorporate higher proportions of forages from other functional groups such as trees and shrubs into their diets (Müller et al., 2012). Tree foliage could represent a potential high quality feed supplement for ruminants (Leng, 1997). However, in these regions a lot of woody forages are rich in plant secondary metabolites (PSM; reviewed by Alonso-Díaz et al., 2010). Although effects might also be positive (Villalba et al., 2010), PSM could have severe side effects on animal health and performance and even be toxic when consumed in excessive amounts (e.g., Bandarra et al., 2011a). Therefore animals either need to be experienced with these plants to avoid the ingestion of detrimental dosages or need to have the possibility to dilute the harmful substances by ingesting alternative feed sources (diet mixing; Duncan et al., 2003). This could be accomplished either by plants low in PSM or by combining plants with different kinds of PSM which are detoxified by different metabolic pathways or act on the base of complementarity (Rogosic et al., 2007; Alonso-Díaz et al., 2010). Therefore both experience and the possibility to choose among several feeds might help animals to overcome negative side effects. The possibility to choose among various feeds of different forage quality might enable individual animals to optimize amounts and complementarity according to their individual nutritional requirements (Provenza et al., 2007). Adult lactating sheep that had the possibility to gain previous experience with various unknown woody plants, showed overall no difference in intake pattern as compared to inexperienced animals (Meier et al., 2013). However, experience gained early in life might be more important and more persistent (Distel et al., 1996).

The objectives of the present study were thus to test (i) if previous experience with novel woody forages with different amounts of PSM (here: phenols) has an effect on feed intake and feeding behavior in growing lambs and (ii) if a diversity of woody forage on offer compared to different single control feeds positively influences feed intake and feeding behavior. The experiment was divided into three test periods and conducted in the semi-arid region of Northeast Brazil using local woody plants as test feeds and growing Santa Inês lambs as experimental animals.

2. Material and methods

2.1. Study region

The study took place at the Federal University of Piauí (UFPI) in Teresina (PI), Brazil (5°5'21"S, 42°48'6"W). It was conducted from early October 2010 to early January 2011. The average temperature during this time was 30°C and the average humidity was 69%. The experiment was designed to meet the 'International Guiding Principles for Biomedical Research Involving Animals' as issued by the Council for International Organizations of Medical Sciences and the International Council for Laboratory Animal Science. The design of the study took into account feed, animal, and set-up specific aspects known to be important for choice feeding experiments (Meier et al., 2012).

2.2. Test feeds and basal diet

Leaves and small twigs from five woody plants native to the study region, which are known to be eaten by sheep, were chosen as test feeds and collected from the experimental farm of UFPI during the dry season 2010 (August–October). Test plants included the semi-deciduous

trees *Cenostigma macrophyllum*, *Combretum leprosum*, *Thiloua glaucocarpa*, and *Trema micrantha* and the evergreen palm tree *Orbignya phalerata* (syn. *Attalea speciosa*). The plant material was dried in the shade and chopped to a maximal size of 1 cm. The woody forages were lower in neutral detergent fiber (NDF) and higher in acid detergent lignin (ADL) than the Bermuda grass hay (Table 1). The crude protein (CP) content was low in the woody plants (≤ 100 g/kg dry matter (DM)), except for *T. micrantha* (165 g/kg DM) which had a higher CP content than the good quality hay (120 g/kg DM). High total phenol (TP) and total tannin (TT) contents were found in *C. macrophyllum*, *C. leprosum*, and *T. glaucocarpa* (180–210 g TP and 165–185 g TT/kg DM). *T. micrantha* was much lower in TP and TT content than the other test plants. Contents of condensed tannins (CT) ranged from 2–240 g/kg DM being lowest in *C. macrophyllum* and the hay and highest in *C. leprosum*, followed by *O. phalerata* and *T. glaucocarpa*. The apparent contradiction of occasionally higher CT than TT contents is likely due to different equivalents used in the analysis.

In addition to the test plants, a basal diet was fed which consisted of locally purchased chopped Bermuda grass hay (*Cynodon dactylon*; hybrid 'Tifton-85') and a concentrate. All animals had always access to hay ad libitum, with an exception of the time when one of the treatment groups received one or all test plants (i.e., the woody plants). Thus, each animal had always access to feed, being either hay or the test plants. Concentrate was offered at an amount of 16 g/kg BW. This was assumed to be 0.40 of the total DM intake (DMI) of the animals which was assumed to be 40 g/kg of body weight (BW) following NRC (1985). In order to account for BW changes, BW weight was measured at the beginning and at the end of each experimental sub-period (in Test period 1 and 2) respectively test period (in Test period 3) and concentrate allocations were adjusted accordingly to BW measured at the beginning. The concentrate was mixed from corn and soybean meal (3:1). A mineral premix (Poli-Sal Ovinos 56, Poli-Nutri Alimentos, Eusébio, Ceará, Brazil) was added to the concentrate (10 g/kg) (Table 1).

2.3. Experimental animals and housing management

Twelve growing Santa Inês lambs were purchased from a farm in São José do Divino (about 180 km north of Teresina). At this farm they had been fed after weaning, which took place at 2 months of age, indoors with elephant grass (*Pennisetum purpureum*) and a concentrate similarly composed as the one used in the experiment. As the lambs were never kept outside, they had no possibility to gain experience with woody forages. The mothers had been kept on an improved grass pasture of *Pennisetum ciliare* and *Brachiaria decumbens* and thus had not obtained experience with woody forage either. At the beginning of the experiment the animals were approximately 6 months old and had a BW of 17 ± 3.2 kg. Animals were kept individually in pens of a floor size of 2×1 m which were located in an open barn. The floor was covered with wood chips made from palm trees. It was controlled by observations that the animals did not eat from this bedding material. The pens were equipped with a water trough in the back and six feeding troughs in the front. Panels between the pens prevented the individual animal from having contact with peers. This arrangement excluded a possible bias in feed selection due to social influence, but conclusions are therefore also restricted to this setting. Test feeding always started at 0800 h in the morning, and test plant(s) or control hay were offered for 6 h according to the experimental design. Concentrate was always provided shortly after this 6 h period as one daily portion. Before starting the test feeding, feed and water troughs were cleaned and water was refilled. After the 6 h of test feeding, the feeds were removed, refusals

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