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# Sheep Preference for Fresh Leaf and Stem of Seven Accessions of Tedera Was Not Influenced by Prior Grazing Experience and Wilting Made No Difference

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**Abstract:** Tedera is a valuable high-quality forage for sheep during summer–autumn. There is evidence that prior grazing experience of novel forages influences preference and haymaking of tedera improves preference by goats. In the first experiment, it was hypothesised that the voluntary feed intake (VFI) of fresh leaves and stems of tedera by sheep would be greater for experienced vs. naïve sheep. In the second experiment, it was hypothesised that the VFI of naïve sheep fed wilted leaves and stems of tedera would be greater than fresh leaves and stems of tedera. To test these hypotheses, adult Merino sheep were fed seven accessions of tedera, in two outdoor pen feeding experiments conducted consecutively. Each of six pens had 14 feeders, two for each accession, and two sheep. In experiment 1, three pens had sheep that had previously grazed tedera (experienced) and three pens had sheep with no experience (naïve), and all were fed fresh leaves and stems of the seven tedera accessions. Experiment 2 involved only naïve sheep, with three pens fed fresh leaves and stems and three pens fed wilted leaves and stems of the seven tedera accessions. Preference was measured each day for six days in experiment 1; and for five days in experiment 2 by calculating the average differences of feed offered and feed remaining from the feeders. In experiment 1, experienced sheep showed no difference in preference (average percentage eaten) in the first hour of each day compared to naïve sheep (70% vs. 56% DM intake (kg), $p = 0.27$). There was an increase in the amount eaten from the first to the last day for both groups, except for the intake of one accession that was reduced for the experienced sheep. In experiment 2, there was no statistical difference in preference between accessions. However, when the average percentage eaten by the experienced and naïve groups are combined, they are strongly correlated, with significant differences between accessions. Sheep did not eat more wilted tedera compared to fresh, which did not support our hypothesis. The only differences we found in sheep preference for tedera accessions occurred in experiment 1. Further studies to investigate seasonal differences in sheep preference between accessions is required and increased replication is needed to better determine the effect of prior grazing experience on the preference for tedera accessions.

**Keywords:** grazing; feed-gap; forage; plant secondary compounds; post-ingestive effects

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## 1. Introduction

The summer and autumn feed gap is a key limitation for improving livestock productivity in regions of Australia characterized by a Mediterranean climate [1]. To fill this summer–autumn feed gap, potential new forage species were sought through an extensive, worldwide search for perennial forage legumes that would suit the Mediterranean-type environments typical of much of southern Australia [2]. Tedera (*Bituminaria bituminosa* var. *albomarginata* and var. *crassiuscula*) was one outstanding candidate identified, and its potential value for Australian livestock production has been confirmed [3]. It has the ability to fill the summer–autumn feed-gap [3,4]; has minimal leaf shedding during summer and autumn [1,3]; is drought and heat tolerant [1,3]; has high nutritional value [5,6]; has an ability to recruit seedlings in autumn within established stands, and contributes to the perenniality of the sward [3,4]. Therefore, tedera appeared to be an ideal option as a legume forage suited to this role. Initially, some 50 accessions of tedera were imported and agronomic studies in a range of locations were used to screen them to select the best parents for breeding purposes [7]. Preference for, or the willingness of sheep to eat a novel plant is an important characteristic in determining the value of the plant as forage for livestock [8,9]. In two previous experiments, Merino sheep that had never eaten tedera (naïve), when given a choice, grazed some accessions more than others, but, when provided enough time, they ate all of them [7,10]. However, the results from these studies remain ambiguous with respect to the effect of initial preference on the long-term intake of forages. In one study [11], preference was assumed to be responsible for observed differences in intake, but, in others, no relationship was found between preference for forage and intake when the choice between forages was removed [12]. Furthermore, the intake of a novel forage by ruminants may be confounded by other dietary and environmental factors [13], for example the quality of their prior diet, such that the level of organic matter delaying the passage of the rumen digesta and the pool of energy available may be limited.

Taste and smell are components of preference by ruminants when selecting foods that meet their nutritional needs and avoiding foods that cause toxicosis [14]. Volatile compounds from lucerne hay have been shown to explain preferences by horses that differ from those predicted from nutritive value [15]. In a similar way, secondary compounds and their interaction with taste and smell may be implicated with the different levels of preference of forages [14,16]. In tedera, wilting has been shown to change preferences by goats [17]; and for *Rhagodia preissii* (Moq.) by sheep [18].

Prior experience of eating a novel forage such as tedera has been shown to significantly increase the intake of the feed by sheep at their next exposure, highlighting the important role of behavioural processes in the determination of food intake [18,19].

In these experiments, our aim was to measure the VFI in order to assess differences in preference among the seven different accessions of tedera tested by Oldham, Real, Bailey, Thomas, Van Burgel, Vercoe, Correal and Rios [10] in an outdoor pen feeding experiment or between wilted versus freshly cut tedera. The experimental layout using outdoor pens aimed to remove external factors associated with outdoor grazing studies such as the effects of other feed types; fixed positions of gates, water trough, sheep yards and handling equipment; paddock shape, slope and shelter; that could influence the VFI of sheep to the seven accessions of tedera.

The experimental approach was similar to that used previously by Ventura, Castanon and Mendez [17]. The study comprised two separate experiments, run consecutively exploring the effects of prior experience and wilting on the preference and VFI of sheep.

In the first experiment, it was hypothesised that the VFI of fresh leaves and stems of tedera by sheep would be greater for experienced vs. naïve sheep. In the second experiment, it was hypothesised that the VFI of naïve sheep fed wilted leaves and stems of tedera would be greater than fresh leaves and stems of tedera.

## 2. Materials and Methods

All procedures reported in this paper were conducted according to the guidelines of the Australian Code of Practice for the Use of Animals for Scientific Purposes [20] and received approval from the Department of Primary Industries and Regional Development (DPIRD) Animal Ethics Committee (AEC 2–12–06).

*2.1. Site Description*

The two outdoor pen experiments were conducted in May 2014 at "Bidgerabbie" farm (Lat: S 30:49:54.393; Long: E 115:45:50.609), Dandaragan, Western Australia. At the site of this outdoor pen feeding experiment, there was an established 1.4 ha area of the seven tedera accessions (Figure 1) [10], growing in individual plots established with an experimental cone-seeder in 2011. The soil on which the tedera accessions were growing at Dandaragan was a deep red sand with $pH_{(CaCl_2)}$ 5.0 (WA Soil Group 445) [21].

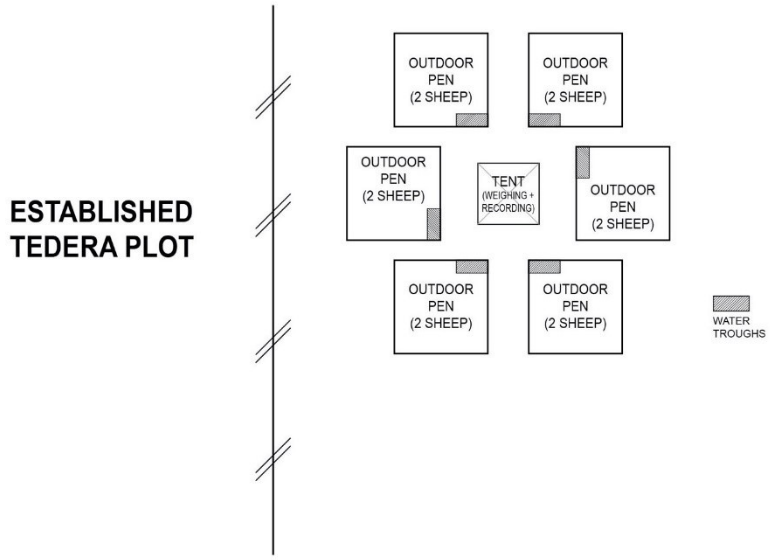


**Figure 1.** Schematic of the pen feeding experiment illustrating the layout of the six pens, the central tent for recording measurements and proximity to the established tedera.

*2.2. Climate*

The site has a historical rainfall average of 460 mm per annum. Monthly rainfall, maximum air temperature and minimum relative humidity data for April, May and June 2014 at the site were measured with automated Department of Primary Industries and Regional Development (DPIRD) weather stations (AWS Junior, MEA 104, Australia) located on site (Table 1).

**Table 1.** The actual rainfall, air temperature and relative humidity data from April 2014 to June 2014 at the Dandaragan experimental site.

| Month | Min. Air Temperature (°C) | Av. Air Temperature (°C) | Max. Air Temperature (°C) | Min. Relative Humidity (%) | Av. Relative Humidity (%) | Max. Relative Humidity (%) | Rainfall (mm) |
|---|---|---|---|---|---|---|---|
| April 2014 | 5.6 | 19.4 | 37.0 | 13.7 | 61.9 | 98.7 | 67.2 |
| May 2014 | 6.0 | 19.7 | 25.6 | 34.8 | 82.4 | 100 | 93.0 |
| June 2014 | 0.5 | 11.9 | 25.4 | 23.2 | 76.0 | 100 | 66.8 |

*2.3. Experimental Design and Treatments*

Two outdoor pen feeding experiments were run consecutively using the same six pens (Figure 1) and the same pen layout with 14 feeders per pen (Figure 2), two for each of the seven accessions. Two sheep were allocated to each pen. For the first of the two outdoor pen experiments (hereafter referred to in this manuscript as experiment 1), three pens had experienced sheep that had previously grazed tedera, and three pens had naïve sheep from the same flock but with no prior experience of grazing tedera. All pens in experiment 1, sheep were offered fresh tedera to eat daily. In the second outdoor pen experiment (hereafter referred to in this manuscript as experiment 2), all sheep were naïve, but sheep in three pens were offered fresh tedera to eat, while sheep in the other three pens were offered wilted tedera. Experiment 1 ran for six days, and experiment 2 ran for five days.

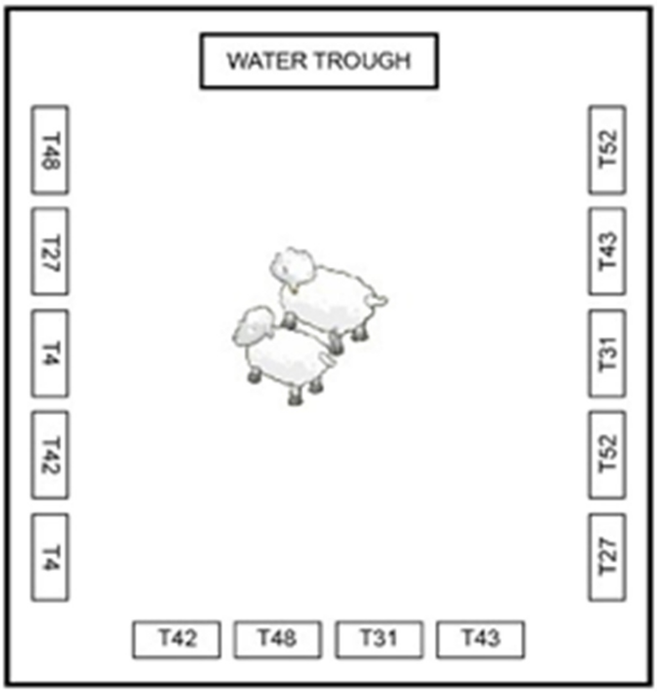


**Figure 2.** Schematic of one of the six 3 m × 3 m outdoor feeding pens illustrating the layout of the 14 feeders with indicative positions of the seven tedera accessions.

*2.4. Forage Material and Its Preparation*

The tedera forage used in the two outdoor pen feeding experiments was collected from a source of established and managed monoculture plots of each of the seven accessions of tedera located close to (50 to 150 m) the experimental feeding pens (Figure 1).These accessions were T4, T27, T31, T42, T43, T48 and T52, and their description can be found in Real, Oldham, Nelson, Croser, Castello, Verbyla, Pradhan, Van Burgel, Méndez, Correal, Teakle, Revell and Ewing [7]. Tedera (leaf and stem) was hand harvested approximately one hour before the start of feeding each day, to a uniform 50–70 mm length to aid intake and minimise wastage (Figure 3).

The plots of tedera had all been preconditioned by grazing followed by mowing to a uniform height for two reasons; firstly, to allow for a uniform starting growth point for all accessions needed for both pen feeding experiments, and secondly, to enable the selection of sheep with prior grazing experience needed for experiment 1.

Each accession was grazed using a grazing pressure (sheep per kg edible dry matter on offer at the start of grazing) calculated to remove 50% of the dry matter (DM) on offer in 14 days. During this grazing, a calibrated visual estimation ($n$ = 30 estimates within each accession), for each accession was used [22] to estimate the tedera DM on offer before and after grazing and the difference between the measures used to estimate the daily intake of tedera by the sheep.

During this precondition period, the sheep grazing each accession were selected at random from a flock of 400 commercial 15-month-old merino wethers. They were double tagged with the same numbered tag to allow permanent identification and for ease of selection for later use in the outdoor pen experiments. The sheep grazing each accession were weighed and condition scored weekly during grazing to estimate their rate of growth during grazing. After grazing, the tedera plots were mown to a uniform height of 10 cm and then allowed to regrow for 60 days before the start of the pen feeding experiments.


**Figure 3.** (**A**) 800 g of fresh tedera from a single plant before harvesting; (**B**) the same tedera plant following harvesting; (**C**) harvesting length (50–70 mm); and (**D**) fresh tedera sample.

Eleven days prior to the commencement of experiment 2, 15 kg of fresh leaves and stems of each tedera accession was cut to a uniform 50–70 mm length. It was allowed to dry over grating in a shed to provide sufficient uniform wilted forage (55% average water content) for experiment 2. Fresh tedera used for experiment 2 was hand harvested one hour before commencement of the feeding as for experiment 1.

### 2.5. Sheep Selection

After the precondition grazing, the tagged sheep used were returned to their original flock ($n$ = 400). During the grazing, all seven accessions of tedera were eaten and all groups of sheep, now deemed to be 'experienced' sheep with respect to tedera, had gained live weight (LW) and maintained body condition. However, the estimated VFI (kg DM/hd/d) of tedera and estimated growth rate of the sheep (g/hd/d) did differ between accessions. Of the seven accessions, the group ($n$ = 14) of sheep grazing accession T42 ate an average of 1.3 kg of tedera DM/hd/d and gained 81 g/hd/d. As this was the closest to the overall average, being 1 kg of tedera DM/hd/d and gained 72 g/hd/d of the seven accessions, these sheep were chosen to supply the six sheep with 'prior experience' of grazing tedera for experiment 1. The individual 'experienced' sheep ($n$ = 6) were selected to minimise the variance in live weight and condition score (CS) [23] within the group and were allocated to each of the three pens at random. Similarly, the remaining 18 sheep required for experiment 1 (six naïve sheep) and experiment 2 (12 naïve sheep) were selected from the remainder of the original flock to match the experienced sheep to be used in experiment 1, for LW and CS. They also were allocated to pens at random.

### 2.6. Acclimatisation to New Feed and Feeding Conditions

Prior to the commencement of the outdoor pen experiments, the flock of sheep were grazing dry pasture composed of annual rye grass, subterranean clover and cape weed (>1000 kg DM/ha) supplemented with lupins. To familiarise the experimental sheep to a pen environment, the sheep were enclosed in a large (18 m $\times$ 18 m) sheltered pen for five days prior to the start of experiment 1 with multiple feeders identical to those used in the experiment. They were provided with a complete diet of macro nutrients and trace minerals (Macco Feeds Australia, Williams, WA, Australia) as pellets (90%) mixed with lupins (10%). The pellets had metabolizable energy 9 MJ/kg (ME) and 11% crude protein (CP).

### 2.7. Randomisation

The treatments of naïve vs. experienced sheep (for experiment 1) and fresh vs. wilted tedera treatments (experiment 2) were randomly allocated among the pens and two sheep were randomly assigned to each pen. In addition, for both experiments, two replicates of the seven accessions of tedera were randomly allocated between the 14 feeders in each pen, each day. Each morning, all feeders to be used in all pens were prefilled and mounted on the outside of their pens. This ensured that all feeders were effectively made available to the sheep in all pens at the same time when feeding started.

### 2.8. Relative Preference Tests

The experimental feeding of tedera was conducted once per day starting at about 10:00 a.m. Each day, all feeders were loaded with 250 g of fresh tedera (two feeders per accession) for each of the seven accessions. A total of 3500 g of fresh tedera (~875 g DM) was offered per pen of two sheep per day. This represented about half of the feed required daily to maintain their LW and CS.

To measure VFI, we calculated the average differences of feed offered and feed remaining from the feeders for each accession during 60-min intervals over two hours in experiment 1 and 30-min intervals in experiment 2. At each measurement, all feeders were removed, then weighed, and returned at the same time.

### 2.9. Maintenance Feeding

At approximately 4:00 p.m. each day, the sheep in the pens were fed (in the same set of feeders) sufficient pellets to complete a maintenance ration over a 24-h period. The sheep used in experiment 2 remained in the holding paddock and were fed the maintenance ration for the six days while experiment 1 was being conducted. Experiment 2 began the day after experiment 1 had concluded.

### 2.10. Sheep Measurements

To determine LW change during the experiment, the sheep were weighed, using a Tru-Test loadbar and Tru-Test XR3000 data logger and condition scored at the beginning of the experiments and directly from their pens at the end of the experiments [23].

### 2.11. Nutritive Value

To determine the DM, leaf to stem ratio and nutritive value of the tedera fed, a representative composite sample from both fresh and wilted material, of each accession was collected at the beginning (day 0) and end (day 14) of the experimental period. Samples were oven dried at 60 °C until there was no weight change and the dry matter of the harvested fresh tedera (DM% (Fresh)) of all accessions was determined. Ground oven-dried samples (DM% (Lab)) were analysed using standard wet chemistry techniques by the methods outlined in AFIA (2011) at the New South Wales Department of Primary Industries accredited (NATA) laboratory and also with near-infrared spectrophotometry [24]. This included: crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ash, dry matter digestibility (DMD), organic matter digestibility (DOMD), estimated metabolisable energy content (ME), organic matter (OM), Nitrate-N; fat and non-structural carbohydrates (NSC).

### 2.12. Statistical Analysis

With experiment 1, analysis of variance was used to analyse the treatment effects of accession, sheep experience and their interaction on the percentage of tedera eaten in the first hour. The analysis was done using pen as the experimental unit for sheep experience and type of tedera. The low degrees of freedom (4) meant that only a very large differences would be detectable as statistically significant for these treatments. Analysis of variance was done for individual days, the average across all days, and as repeated measures. Experiment 2 was analysed in a similar way with tedera type (fresh and wilted) replacing sheep experience. Analysis of variance was used to analyse any differences in nutritive value between tedera accession. The analysis was done using day 0 and day 14 as replicates. All analyses were conducted using Genstat version 20. Significance refers to statistical significance at a 95% confidence level ($p < 0.05$), unless otherwise indicated.

## 3. Results

### 3.1. General

Sheep maintained LW (average 51 kg) and CS (average 3.3) during the two experiments, and there were no differences in sheep performance. The analysis of nutritive value and leaf–stem ratio of the tedera fed during the two experiments (using wet chemistry and near-infrared spectrophotometry) is shown in Table 2. There were significant differences ($p$-0.001) between accessions for DM% (Fresh) and ($p$-0.034) for DM% (Lab). The seven accessions of tedera were similar in all other measures of nutritive value.

**Table 2.** Nutritive value of the seven accessions of tedera fed. Analysis of the representative composite samples collected at days 0 and 14 of the experimental period. (a) Leaf %, leaf to stem ratio; DM% (Fresh), dry matter of the harvest fresh tedera; DM% (Lab), dry matter after oven drying; NDF (%), neutral detergent fibre; ADF (%), acid detergent fibre; CP (%), crude protein; ME (%), estimated metabolisable energy; (b) ASH (%), ash; OM (%), organic matter; DMD (%), dry matter digestibility; DOMD (%), organic matter digestibility; ADL (%), acid detergent lignin; Nitrate-N (%); Fat (%); and NSC (%), non-structural carbohydrates.

| **Accessions** | **Leaf%** | **DM% (Fresh)** | **DM% (Lab)** | **NDF** | **ADF** | **CP** | **ME** |
|---|---|---|---|---|---|---|---|
| T4 | 85.0 | 22.3 | 96.6 | 41.0 | 27.2 | 17.8 | 9.8 |
| T27 | 92.6 | 23.1 | 97.4 | 33.7 | 24.2 | 18.2 | 10.6 |
| T31 | 82.6 | 26.6 | 97.2 | 40.3 | 27.4 | 16.0 | 9.8 |
| T42 | 82.2 | 25.9 | 95.9 | 31.9 | 22.5 | 18.5 | 10.7 |
| T43 | 82.0 | 28.3 | 97.4 | 37.9 | 27.6 | 16.2 | 10.0 |
| T48 | 85.5 | 25.8 | 96.3 | 38.8 | 25.8 | 16.2 | 10.5 |
| T52 | 82.8 | 24.2 | 97.4 | 43.4 | 27.9 | 17.2 | 10.2 |
| $p$-value | 0.359 | 0.001 | 0.034 | 0.182 | 0.431 | 0.158 | 0.811 |
| LSD | 11.13 | 2.27 | 0.9 | 9.6 | 6.5 | 2.3 | 1.9 |

| **Accessions** | **ASH** | **OM** | **DMD** | **DOMD** | **ADL** | **Nitrate-N** | **FAT** | **NSC** |
|---|---|---|---|---|---|---|---|---|
| T4 | 11.1 | 88.9 | 66.3 | 63.0 | 13.5 | 96.5 | 3.1 | 27.1 |
| T27 | 10.9 | 89.1 | 71.1 | 67.1 | 12.6 | 112.5 | 3.1 | 34.2 |
| T31 | 10.3 | 89.7 | 66.6 | 63.2 | 12.1 | 60.5 | 3.3 | 30.2 |
| T42 | 10.1 | 89.9 | 71.7 | 67.5 | 13.2 | 87.5 | 2.9 | 36.7 |
| T43 | 9.9 | 90.1 | 67.5 | 64.0 | 10.4 | 69.0 | 3.0 | 33.1 |
| T48 | 9.9 | 90.1 | 70.5 | 66.6 | 10.4 | 82.0 | 3.0 | 32.1 |
| T52 | 10.0 | 90.0 | 68.4 | 64.8 | 12.8 | 125.0 | 3.0 | 26.4 |
| $p$-value | 0.349 | 0.349 | 0.811 | 0.811 | 0.139 | 0.213 | 0.694 | 0.152 |
| LSD | 1.4 | 1.4 | 11.2 | 9.5 | 2.8 | 56.3 | 0.5 | 8.3 |

### 3.2. Experiment 1

Over the six days of the experiment, the experienced sheep ate 70% of the tedera in the first hour and 86% after two hours, whereas the naïve sheep ate 56% and 75%, respectively. The difference between the two groups was not significant ($p = 0.27$; $p = 0.37$), with both groups showing an increase in the amount eaten over successive days of the experiment (Figure 4).

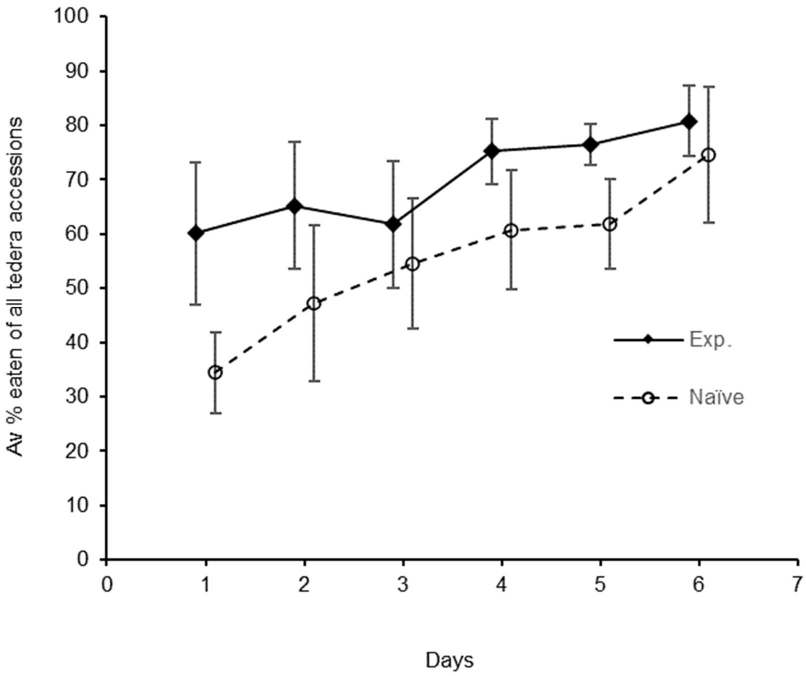


**Figure 4.** The average percentage of the tedera offered that was eaten in the first hour by both the experienced (diamond) and the naïve (circle) sheep over the six days of experiment 1.

The experienced and naïve sheep ate more of all the accessions of tedera on day six compared to the first day, except for the consumption of accession T4 by the experienced sheep. With accession T4, the experienced sheep expressed an aversion to eating it for the first three days after which the amount eaten each day started to increase but remained much lower than the average intake of the remaining six accessions throughout (Figure 5). While the repeated measures analysis only pointed to significant accession and time effects ($p < 0.001$) but no significant time by accession interaction ($p = 0.33$) or time by treatment (experience) ($p = 0.21$), analysis of change over time did show significant accession differences with T4 significantly different from most other accessions ($p < 0.001$). Although the intake of naïve sheep increased over time, it was not significant ($p = 0.42$).

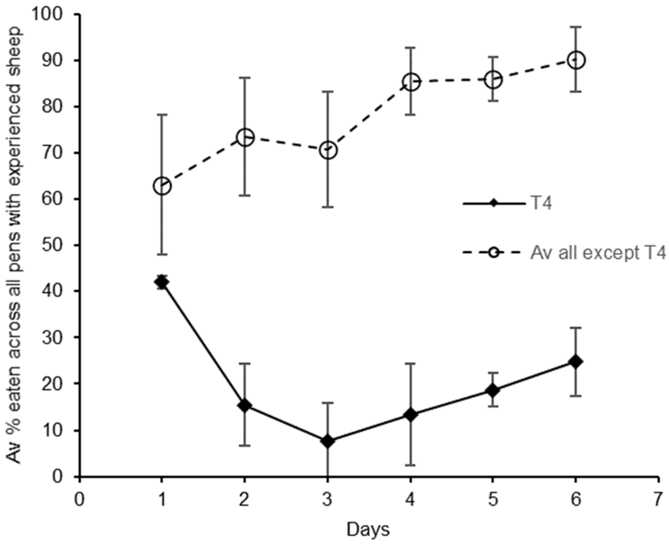


**Figure 5.** The combined average percentage eaten in the first hour of six tedera accessions (circles) offered in contrast with the average percentage eaten of tedera accession T4 (diamonds), by the experienced sheep over the six days of experiment 1. Note: Error bars are standard errors.

Excluding accession T4, there was no significant accession by experience interaction and high correlation between experienced and naïve for the average percentage eaten after one hour (r = 0.96). The VFI of the two groups of sheep are strongly associated. However, there were significant differences between accessions ($p < 0.001$) for the average percentage eaten for both experienced and naïve sheep combined (Table 3). There was no correlation (r = 0.41) between sheep preference and the DM% (Fresh).

**Table 3.** Overall preference ranking of all accessions in experiment 1 for the first hour of feeding over six days. Accessions that share no common letters are significantly different ($p < 0.05$; LSD 11).

| Accession | Average % Eaten | Significance | Ranking |
|---|---|---|---|
| T4 | 36 | c | 7 |
| T27 | 78 | a | 1 |
| T31 | 53 | b | 6 |
| T42 | 77 | a | 2 |
| T43 | 73 | a | 3 |
| T48 | 61 | b | 4 |
| T52 | 60 | b | 5 |

The intake of tedera accession T42 that they had previously grazed exclusively (60 days earlier) was high, although it remained no different from accessions T27 and T43. However, this group of sheep responded with an aversion to eating T4 ($p < 0.001$).

*3.3. Experiment 2*

Results from the first day of experiment 2 were excluded because a rainfall event affected measurement of intake. Over the four remaining days of experiment 2, the average amount eaten in the first hour was 71% for both fresh and wilted tedera. For both treatments, there was an increase in the amount eaten over time (Figure 6).

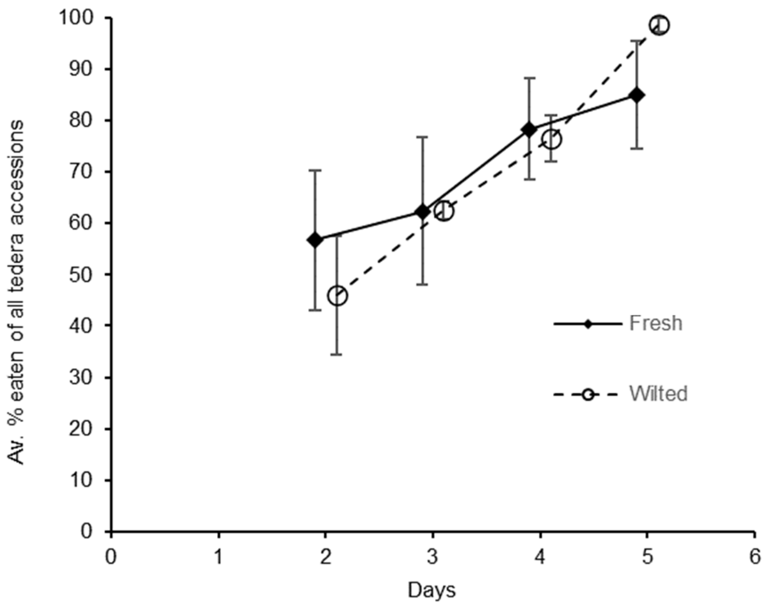


**Figure 6.** The average percentage of the tedera offered that was eaten in the first hour by naive sheep fed fresh (diamond) and wilted (circle) tedera over four days of experiment 2. Note: Error bars are standard errors.

Unlike experiment 1, there was no significant difference in preference between accessions. Sheep ate an average 72% of accession T42, which was similar to the overall average. Looking only at the naïve sheep fed fresh tedera (three pens in both experiments had this same treatment), the correlation between experiments for the percentage of each accession eaten was 0.37. As such, the VFI of the naïve sheep fed fresh tedera over time is similar between experiments.

**4. Discussion**

Our resultsdid not support the first hypothesis, that the voluntary feed intake will be greater for sheep that had prior experience of grazing tedera relative to the voluntary feed intake of naïve sheep. In experiment 1, there was a general increase in the intake of tedera for both experienced and naïve groups of sheep over the experimental period, and a significant time effect. Sheep are known to take time to adjust and accept a novel feed [25] and to a novel environment [13] as the sheep in our study may have still been becoming familiar with the new feeding pens. The experimental pen design aimed to reduce external factors influencing grazing behaviour, thereby affecting intake; however, only two sheep per pen may have had a contradictory affect. Penning [26] found that a minimum flock size is three [18], preferably four sheep for grazing behaviour studies. This is consistent with the concept that forage intake is a learning process for sheep, whether selected as a monoculture or in diverse pastures, the preference thereafter being regulated by the experiences the sheep have had with the food's post-ingestive effects [27].

This delayed rate of voluntary feed intake by the naïve sheep to the tedera accessions may be explained by its neophobic factors similar to sheep fed the novel woody forage shrub, *Rhagodia preissii* (Moq.) [18]. Here, these limitations were overcome by a familiarisation process comprised of training. Whilst it did not change preference, it did increase intake in subsequent feeding though the effect of the training did vary greatly between individual sheep. Similarly, naïve sheep in a previous study showed preference between two accessions of tedera and lucerne where there was a clear preference for one accession of tedera over another on subsequent exposure [19]. Further studies in Israel investigating different grazing intensities by cattle on aboveground biomass found that increasing grazing intensity and a history of previous grazing affected the performance of tedera [28]. Given that there was no difference in sheep grazing preference between experience and naïve sheep, the advantage of training is not supported.

The voluntary feed intake of both experienced and naïve groups when combined were strongly associated with significant differences between accessions for the average percentage eaten. This is a useful finding for breeders and selection of tedera accessions to be progressed to commercialisation.

In experiment 1, the sheep with the prior experience of having eaten tedera (accession T42) responded with an aversion to now eating accession T4. This difference is not explained by standard analysis of factors driving nutritive value. The aversion response may represent a transient integration of factors resulting from eating the fresh tedera, including secondary compounds combined with aversive behaviour from a grazing experience prior to the experiment [29]. Provenza, Villalba, Dziba, Atwood and Banner [27] contend that transient food aversions, based on prior grazing experience, limit intake of single-food diets when given choice even when they meet the nutrient requirements. The presence of, or more elevated concentration of secondary compounds, perhaps being greater and more persistent for T4 than the other accessions. Furthermore, despite having previous experience, albeit a different accession (T42), the experienced sheep preference for the remaining six accessions was similar. The choice of T42 sheep as having 'experienced' grazing tedera was justified as they showed no bias towards this accession. The strong aversion by the experienced sheep to accession T4 corresponds well to the post-ingestive feedback actions of the rumen [30]. Although only for the first three days, it seems plausible that the experienced sheep when considering T4 received avoidance dietary/sensory cues likely to be in anticipation of an unfavourable consequence from its consumption as well as a dislike to its flavour [31], a mechanism that is enabled by the organoleptic sensory system in ruminants [32].

Another possible causal factor is the physical cutting from the nearby tedera plots. Changes in chemical classes following defoliation could have produced the volatile secondary compounds in accession T4 and that the experienced sheep associate with their previous grazing experience. The odour is known to be the result of a pool of substances and not a single odour impression [33]. These chemical classes may vary between and within species, in this case tedera accessions, and influenced by the environmental conditions and grazing interactions [34].

Furthermore, the variation between individual animal responses is important, as the individuals have been highly selective, though the selectively of the flock as a whole will be much less because of the range in individual preferences [18,35].

Sheep adopt different foraging strategies in response to changing forage characteristics, increasing their preference for legume plants that increase their intake of digestible dry matter as mixed pastures mature [36]. This is unlikely to have been a factor in our experiment as, with the exception of dry matter (DM% Fresh; DM% Lab) content, there were no significant differences in nutritive value between the accessions offered. As such, nutritive value is unlikely to be a preference cue as the tedera offered was 50% of the diet and the experimental grazing period was less than a week. Acceptability factors other than nutritive value are more likely to be at play as sheep sensory receptors operate at a molecular level and do not respond to varying proportions of proteins, soluble carbohydrate, fat and fibre [35].

In an outdoor grazing experiment, and with the same seven tedera accessions, Ref. [10] also found a positive preference for T43 though a negative preference for T42 and T27. This variation indicates that secondary compounds are likely to be a factor in the preference differences of these accessions. This requires methods to more quantitatively measure the presence and abundance of secondary compounds that might be operating as attractant or repellent cues to grazing ruminants [15].

The second hypothesis that the VFI fed in pens of wilted leaf and stem would be greater than fresh leaves and stems of tedera was rejected. There appears to be no plant secondary compounds removed by drying that are inhibiting the voluntary feed intake. This is a different result to experiences reported by Ventura, Castanon and Mendez [17]; no other study has reviewed preference for tedera hay by sheep. Future studies investigating the effect of wilted tedera with varying components of seed and fruit on sheep preference should be considered.

The results from the two experiments highlight the findings of previous studies investigating intake of novel forage shrubs confirming a high degree of variability among individual animals [11]. The outdoor pen experimental approach was effective, but, in the future, modifications to increase the number of sheep to counteract the variability effect of individual sheep should be considered. During the lifetime of an individual animal, its preference for forage is acquired by its own experience through the post-ingestive effects of the food and trans-generational (social) learning from the flock [37]. As such, being a novel forage species, the individual experience is the determining factor and a larger sample size is necessary. Modifications might include increasing the number of pens; reducing the number of feeders; and comparing fewer accessions.

Given that preference among sheep for components of pasture mixes are influenced by the plant nutritive characteristics as plants mature, it would be beneficial to further investigate the seasonal effects on sheep preference of all tedera accessions. To avoid cutting and collecting tedera for feeding in pens, consideration should be for the preference testing methodology to be undertaken in grazing conditions or from pots if pens are used. A next step is to identify the volatile compounds that might be influencing the variability in preference of tedera [38,39]. We then need to preference test responses of sheep to the taste and/or smell of known volatile compounds and their concentrations to identify which adversely or favourably affect the feeding behaviour, [35]. This could be accomplished by spraying combinations of these volatiles on a given forage, using paired testing and evaluating animal preference for the variously treated diets [32].

A management approach to offset the effects of plant secondary compounds, particularly in summer, on sheep preference is to remove diet choice by growing tedera in dense stands in rotational paddocks, grazed at moderate to high intensities in short periods, from early summer through to late autumn [28]. Another proven approach is to graze with moderate grazing intensities with set-stocking [3]; here, naïve sheep have time to adjust to the novel feed. Despite not having been able to confirm the effect of prior grazing experience, a precautionary approach to both systems could be augmented with the inclusion of experienced sheep in the flock and thereby helping assure the utilisation of quantities of high quality feed for maintaining or finishing sheep in late summer and autumn when it is of most economic value [4].

## 5. Conclusions

The hypothesis that voluntary feed intake will be greater for sheep that had prior exposure to grazing tedera relative to the voluntary feed intake of naïve sheep is not supported. Overall, both groups increased the amount eaten over successive days, suggesting that intake was also affected by acclimation to the new environment.

The voluntary feed intake of both experienced and naïve groups when combined were strongly associated with significant differences between accessions for the average percentage eaten. Further studies to investigate the seasonal differences in sheep preference between accessions is required in addition to identifying the volatile compounds and their concentrations in the different tedera accessions.

The second hypothesis that the voluntary feed intake of wilted leaf and stem would be greater than fresh leaves and stems of tedera was rejected. Further studies that compare relative preference by sheep for wilted and fresh tedera accession at the seed maturity stage of tedera growth are required.

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## References

1. Moore, A.D.; Bell, L.W.; Revell, D.K. Feed gaps in mixed-farming systems: Insights from the Grain & Graze program. *Anim. Prod. Sci.* **2009**, *49*, 736–748.
2. Real, D.; Li, G.D.; Clark, S.; Albertsen, T.O.; Hayes, R.C.; Denton, M.D.; D'Antuono, M.F.; Dear, B.S. Evaluation of perennial forage legumes and herbs in six Mediterranean environments. *Chil. J. Agric. Res.* **2011**, *71*, 357–369. [CrossRef]
3. Real, D.; Oldham, C.M.; van Burgel, A.; Dobbe, E.; Hardy, J. Tedera proves its value as a summer and autumn feed for sheep in Mediterranean-like climates. *Anim. Prod. Sci.* **2018**, *58*, 2269–2279. [CrossRef]
4. Finlayson, J.; Real, D.; Nordblom, T.; Revell, C.; Ewing, M.; Kingwell, R. Farm level assessments of a novel drought tolerant forage: Tedera (Bituminaria bituminosa C.H. Stirt var. albomarginata). *Agric. Syst.* **2012**, *112*, 38–47. [CrossRef]
5. Ghaffari, M.H.; Durmic, Z.; Real, D.; Vercoe, P.; Smith, G.; Oldham, C. Furanocoumarins in tedera do not affect ruminal fermentation in continuous culture. *Anim. Prod. Sci.* **2015**, *55*, 544. [CrossRef]
6. Oldham, C.M.; Wood, D.; Milton, J.; Real, D.; Vercoe, P.; van Burgel, A.J. An animal house study on utilisation of fresh tedera (*Bituminaria bituminosa* var. *albomarginata* and *crassiuscula*) by Merino wethers. *Anim. Prod. Sci.* **2015**, *55*, 617–624. [CrossRef]
7. Real, D.; Oldham, C.M.; Nelson, M.N.; Croser, J.; Castello, M.; Verbyla, A.; Pradhan, A.; Van Burgel, A.; Méndez, P.; Correal, E.; et al. Evaluation and breeding of tedera for Mediterranean climates in southern Australia. *Crop Pasture Sci.* **2014**, *65*, 1114–1131. [CrossRef]
8. Rossiter, R.C. Ecology of the Mediterranean Annual-Type Pasture. *Adv. Agron.* **1966**, *18*, 1–56.
9. Masters, D.G.; Mata, G.; Revell, C.K.; Davidson, R.H.; Norman, H.C.; Nutt, B.J.; Solah, V. Effects of Prima gland clover (*Trifolium glanduliferum* Boiss cv. Prima) consumption on sheep production and meat quality. *Aust. J. Exp. Agric.* **2006**, *46*, 291–297. [CrossRef]

10. Oldham, C.; Real, D.; Bailey, H.J.; Thomas, D.; Van Burgel, A.; Vercoe, P.; Correal, E.; Rios, S. Australian and Spanish scientists are collaborating in the domestication of tedera: Young Merino sheep grazing a monoculture of tedera in autumn showed preference for certain accessions but no signs of ill health. *Crop Pasture Sci.* **2013**, *64*, 399–408. [CrossRef]
11. Greenhalgh, J.F.D.; Reid, G.W. Relative palatability to sheep of straw, hay and dried grass. *Br. J. Nutr.* **2007**, *26*, 107–116. [CrossRef] [PubMed]
12. Gherardi, S.; Black, J. Effect of palatability on voluntary feed intake by sheep. I. Identification of chemicals that alter the palatability of a forage. *Aust. J. Agric. Res.* **1991**, *42*, 571–584. [CrossRef]
13. Weston, R. Some aspects of constraint to forage consumption by ruminants. *Aust. J. Agric. Res.* **1996**, *47*, 175–197. [CrossRef]
14. Provenza, F.D. Postingestive feedback as an elemnary determinant of food preference and intake in ruminants. *J. Range Manag. 1995b*, 2–17. [CrossRef]
15. Pain, S.; Revell, D. Natural odorants can influence the preference of horses for lucerne hay. In *Proceedings of the Recent Advances in Animal Nutrition in Australia*; Nottingham University Press: Nottingham, UK, 2007; pp. 27–33.
16. Gutman, M.; Perevolotsky, A.; Sternberg, M. Grazing effects on a perennial legume, *Bituminaria bituminosa* (L.) Stirton, in a Mediterranean rangeland. *Cah. Options Mediterraneennes* **2000**, *45*, 299–303.
17. Ventura, M.R.; Castanon, J.I.R.; Mendez, P. Effect of season on tedera ( Bituminaria bituminosa) intake by goats. *Anim. Feed Sci. Technol.* **2009**, *153*, 314–319. [CrossRef]
18. Wallis, R.H.; Thomas, D.T.; Speijers, E.J.; Vercoe, P.E.; Revell, D.K. Short periods of prior exposure can increase the intake by sheep of a woody forage shrub, Rhagodia preissii. *Small Rumin. Res.* **2014**, *121*, 280–288. [CrossRef]
19. Davies, G. Preference and Palatibility of Tedera Varies between Lines and Is Increased by Prior Experience. Ph.D. Thesis, Murdoch University, Perth, Australia, 2010.
20. National Health and Medical Research Council, *Australian Code for the Care and Use of Animals for Scientific Purposes (the Code)*, 8th ed.; Australian Government Publishing: Canberra, Australia, 2013; (updated 2021).
21. Schoknecht, N.R.; Pathan, S. Soil groups of Western Australia: A simple guide to the main soils of Western Australia. *Perth. Rep.* **2013**, *380*, 175.
22. Cayley, J.W.D.; Bird, P.R. *Techniques for Measuring Pastures*; Technical Report 191; Victorian Department of Agriculture: Melbourne, Australia, 1996.
23. van Burgel, A.J.; Oldham, C.M.; Behrendt, R.; Curnow, M.; Gordon, D.J.; Thompson, A.N. The merit of condition score and fat score as alternatives to liveweight for managing the nutrition of ewes. *Anim. Prod. Sci.* **2011**, *51*, 834–841. [CrossRef]
24. Adriansz, T.D.; Hardy, J.L.M.; Milton, J.T.B.; Oldham, C.M.; Real, D. Near infrared analysis for nutritive attributes of tedera (Bituminaria bituminosa var. albomarginata). *J. Near Infrared Spectrosc.* **2017**, *25*, 215–218. [CrossRef]
25. Kenney, P.A.; Black, J.L. Factors affecting diet selection by sheep. 1. Potential intake rate and acceptability of feed. *Aust. J. Agric. Res.* **1984**, *35*, 551–563. [CrossRef]
26. Penning, P.D.; Parsons, A.J.; Newman, J.A.; Orr, R.J.; Harvey, A. The effects of group size on grazing time in sheep. *Appl. Anim. Behav. Sci.* **1993**, *37*, 101–109. [CrossRef]
27. Provenza, F.D.; Villalba, J.J.; Dziba, L.; Atwood, S.B.; Banner, R.E. Linking herbivore experience, varied diets, and plant biochemical diversity. *Small Rumin. Res.* **2003**, *49*, 257–274. [CrossRef]
28. Sternberg, M.; Gishri, N.; Mabjeesh, S.J. Effects of Grazing on Bituminaria bituminosa (L) Stirton: A Potential Forage Crop in Mediterranean Grasslands. *J. Agron. Crop Sci.* **2006**, *192*, 399–407. [CrossRef]
29. Thomas, D.; Milton, J.; Revell, C.; Ewing, M.; Lindsay, D. Individual and socially learned preferences for biserrula (Biserrula Pelecinus L.) in sheep. *Grass Forage Sci.* **2015**, *70*, 374–380. [CrossRef]
30. Fisher, D.S. A Review of a Few Key Factors Regulating Voluntary Feed Intake in Ruminants1. *Crop Sci.* **2002**, *42*, 1651–1655. [CrossRef]
31. Provenza, F.D. Role of Learning in Food Preferences of Ruminants: Greenhalgh and Reid Revisted. In *Proceedings of the Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*; Ferdinand Enke Verlag: Stuttgart, Germany, 1995; pp. 233–247.
32. Mayland, H.F.; Shewmaker, G.E. *Plant attributes that affect livestock selection and intake.*; University of Idaho: Moscow, ID, USA, 1999; p. 6.
33. Tava, A.; Pecetti, L.; Ricci, M.; Pagnotta, M.A.; Russi, L. Volatile compounds from leaves and flowers of *Bituminaria bituminosa* (L.) Stirt. (Fabaceae) from Italy. *Flavour Fragr. J.* **2007**, *22*, 363–370. [CrossRef]
34. Provenza, F.D.; Villalba, J.J.; Haskell, J.; MacAdam, J.W.; Griggs, T.C.; Wiedmeier, R.D. The Value to Herbivores of Plant Physical and Chemical Diversity in Time and Space. *Crop Sci.* **2007**, *47*, 382–398. [CrossRef]
35. Arnold, G.W.; Boer, E.; Boundy, C.A.P. The influence of odour and taste on the food preferences and food intake of sheep. *Aust. J. Agric. Res.* **1980**, *31*, 571–587. [CrossRef]
36. Thomas, D.T.; Milton, J.T.B.; Revell, C.K.; Ewing, M.A.; Dynes, R.A.; Murray, K.; Lindsay, D.R. Preference of sheep among annual legumes is more closely related to plant nutritive characteristics as plants mature. *Anim. Prod. Sci.* **2010**, *50*, 114–123. [CrossRef]
37. Villalba, J.J.; Provenza, F.D. Learning and Dietary Choice in Herbivores. *Rangel. Ecol. Manag.* **2009**, *62*, 399–406. [CrossRef]

38. Oertli, E.H.; Beier, R.C.; Ivie, G.W.; Rowe, L.D. Linear furocoumarins and other constituents from *Thamnosma texana*. *Phytochemistry* **1984**, *23*, 439–441. [CrossRef]
39. Innocenti, G.; Piovan, A.; Filippini, R.; Caniato, R.; Cappelletti, E.M. Quantitative recovery of furanocoumarins from *Psoralea bituminosa*. *Phytochem. Anal.* **1997**, *8*, 84–86. [CrossRef]