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Tannin content of selected fodder trees and shrubs and their effect on in vitro digestibility

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Abstract

Ten fodder trees and shrub species growing in the Mediterranean zone are examined in this paper. Arbutus unedo, Arbutus andrachne, Arbutus andrachnoides a hybrid (Arbutus unedo X Arbutus andrachne), Amorpha fruticosa, Colutea arborescens, Gleditshia triacanthos, Morus alba, Morus alba cv. Kokuso 21, Robinia pseudoacacia, Phillyrea media. Browse samples were harvested in late May (spring stage) and in late July (summer stage). Samples were analysed for total phenolics content (Folin-Denis assay), condensed tannins (vanillin-HCl assay) and their astrigency effect (haemoglobin precipitation assay) as well as for the in vitro dry matter digestibility (IVDMD). Total tannin concentration was more than twice as much from that of alfalfa during the spring stage for all fodder and shrub species tested. The general trend was for decreased total and condensed tannin content with maturation. The astrigency effect though had an increased trend with maturation. Negative correlations were found only between digestibility and astrigency assay in both phenological stages, higher in the Summer than in the Spring.

Introduction

In the Mediterranean zone shrub species and fodder trees comprise a major component of the grazing animals diet. Cell contents and structural constituents as well as secondary chemical compounds are the main factors which affect digestibility and thus can be used as independent parameters (Burns and Cope 1974) for fodder quality evaluation and rates of decomposition.

Secondary chemical compounds exist in a wide variety of plant species. Bate-Smith and Metcalf (1957) have reported that

80% of woody perennial dicots and 15% of annual and herbaceous perennial dicots contain tannins. Secondary chemical compounds especially tannins play a very important role in affecting forage preference and quality. It have been speculated that they are an ecologically developed defence mechanism. Provenza and Malechek (1983) have reported that plant parts which are more succeptible to herbivory damage contain higher concentrations that their counterparts which have been developed at heights beyond the animal reach. Tannins are polyphenols with high molecular weight which can bind to both proteins and carbohydrates. Their binding ability varies according to their chemical structure. They are classed into two major categories, the hydrolyzable and condensed tannins. Hydrolyzable tannins are esters of glucose and phenolic acids, while condensed tannins are polymers of condensed flavan-3-ol units (Burritt et al. 1987). Tannins bind to proteins modifing the rate and extend of their digestion (Feeny 1970). In rodents, food consumption has been found (Joslyn and Click 1969, Tamir and Alumot 1970, Mitjavila et al. 1977) that is depressed, when foods contain relatively high levels of hydrolyzable tannins (5%). The limited intake may result partly also from reduced digestion since condensed tannin fraction seem to depress digestion. The extend of reduced digestion depends on the degree of their polymerization (Martin-Tanguy et al. 1977). Although hydrolyzable and condensed tannins are structurally different, both tannins are capable of forming strong complexes with certain type of proteins (Hagerman and Klucher 1986). However, the effect of condenced tannins in binding affinity is far more pronounced.

Ecological implications of phenolics contained in plant

tissue have been demonstrated (Feeny 1976, Coley 1983, Muller et al 1987) that reduce litter decomposition resulting in formation of thicker humus layers when present and thus affect site quality in the mediterranean ecosystem. The purpose of this study was to investigate the content of different tannin groups in several fodder woody species and their effect on in vitro digestibility.

Materials and Methods

Ten woody species growing in the Mediterranean zone Arbutus unedo L., Arbutus andrachne L., Arbutus andrachnoides LK a hybrid (Arbutus unedo x Arbutus andrachne), Amorpha fruticosa L., Colutea arborescens L., Gleditschia triacanthos L., Morus alba L., Morus alba cv. kokuso 21, Robinia pseudacacia L., Phillyrea media L. Current season's twigs and leaves similar to those grazed, were collected at two phenological stages: When the rapid growth was terminated in late May (Spring stage) and when growth had ceased and stems had hardened in late July (Summer stage). Samples were dried at 40°C and ground through a 40-mesh sieve. For comparative purposes a sample of good quality alfalfa (Medicago sativa L.) was also evaluated. For the above samples two separate tannin assays and a quantitative evaluation of total phenolics were utilized, since they contain a sum of various phenolic compounds and it is not precisely defined which method is more pertinent for their determination (Bullard et al. 1981, Martin and Martin 1982). The Folin-Denis quantitative procedure (Burns 1963, Martin and Martin 1982) was used. This measures total phenolic acids, flavonoids, and tannins and is based on the

reducing power of the hydroxyl groups (Hahn et al. 1984). Results from this method are often expressed in terms of tannic acid equivalents (Martin and Martin 1982). The vanillin-HCl assay (Price et al. 1978) which reacts with certain types of flavonoids, many of which are precursors to condensed tannin but do not precipitate proteins (Waterman et al. 1980). Results from this assay are expressed in catechin equivallents. The astrigency assay (Bate-Smith 1973) expressing the efficiency of tannins as precipitants of protein. It is determined by their reaction with the proteins of haemolysed blood and calorimetric determination of residual haemoglobin. The method is accurate and sensitive because of the narrow range of concentration of tannin between that required to initiate precipitation and that for complete precipitation. Haemanalysis thus, shows promise of being a useful tool in tannin analysis. In addition all samples were processed by the two stage (Tilley and Terry 1963) procedure so that to evaluate their in vitro dry matter digestibility (IVDMD).

The analysis of variance for comparing the tannin concentrations was factorial between the shrub species and phenological stages with 10 and 2 treatments respectively. Significant differences between species means were detected at the 0.05 level using the least significant difference (L.S.D.) criterion for each phenological stage and analytical assay and between the phenological stages.

Results and Discussion

Estimated tannin concentrations depended on shrub species as well as analytical assays (Table 1). No significant correlation

Table 1. Digestibility and tannin levels (mg of equivalents/g) of organic matter in two phenological stages from several fodder trees and shrubs as estimated by 4 assays.

SPECIES	Folin - Denis[1] T.A.E.mg/g		Vanillin - HCl[2] C.E mg/g		Astrigency[3] T.A.E. %		Digestibility (IVDMD) %	
	Spring stage	Summer stage	Spring stage	Summer stage	Spring stage	Summer stage	Spring stage	Summer stage
Medicago sativa (Me. sa)	18.28		8.56		9.33			
Arbutus unedo (Ar. un)	218.15a	150.91b	15.38a	20.92a	9.475a	9.058b	51.3	41.6
Arbutus andrachne (Ar. an)	207.48a	119.57b	15.23a	16.96a	8.766a	8.951b	49.5	42.7
Arbutus andrachnoides (Ar. andes)	194.05a	148.49b	18.93a	24.66b	9.529a	8.512b	46.3	37.77
Phillyrea media (Ph. me.)	94.98a	85.08a	22.41a	10.92b	9.028a	9.228b	48.3	44.97
Morus alba (Mo. al.)	53.33a	18.07b	16.93a	9.73a	9.393a	9.054b	71.20	72.11
Morus alba cv. Kokuso	65.09a	15.30b	13.88a	13.52a	9.139a	8.989b	76.0	75.6
Colutea arborescens (Co. ar.)	46.02a	18.55b	19.20a	11.66b	9.346a	8.951b	71.9	74.02
Gleditschia triacanthos (Gl. tr.)	59.24a	17.55b	14.57a	12.42a	9.235a	9.218a	45.88	41.70
Amorpha fruticosa (Am. fr.)	83.07a	37.79b	25.13a	17.45b	9.145a	9.288b	48.53	44.54
Robinia pseudoacacia (Ro. ps.)	64.96a	19.27b	27.99a	14.59b	8.298a	8.945a	55.89	48.90
L.S.D.	7.1	19.4	1.85	4.2	0.080	0.090		

1. Total phenols 2. Catechin equivalents 3. Tannic acid equivalents
* Means between the columns in each analytical assay followed by the same letter are not significantly different (p= 0.05).

alfalfa test samples. The general trend was for reduced tannin content during the summer than the spring stage. Nastis and Malechek (1981) reported a decrease in tannin content from June to August by 20%. Our results are in agreement also with findings of Reed (1986) testing east African browse species and Burritt et al. (1987) testing woody species.

Astrigency assay

During spring higher astrigency of tannins was observed for the species Ar. un. Ar. andes, followed by the remaining species in the following order Mo.al., Co.ar., Gl.tr., Am.fr., Mo.al.cv., and Ph.me., Ro.ps., Ar.an. (LSD = 0.080). From these species significant reduction ($P \leq 0.05$) was observed in the species Ar. un., Ar. andes, Mo.al., Mo.al.cv., Co.ar., while for the species Am.fr., Ph.me., Ar.an. a significant increase was observed in the summer phenological stage. Our results are in agreement with those reported by Reed (1986) and Burritt et al. (1987).

Digestibility

Dry matter digestibility was higher in both phenological stages for the species Mo.al.cv., Mo.al. Co.ar., and Ro.ps., in comparison with the remaining species. The relationships of tannin concentrations of Folin-Denis, vannilin-HCl and astrigency assays with the dry matter digestibility in each phenological stage were significant only for the astrigency assay in both phenological stages. Tannin concentrations and digestibility were negatively correlated indicating the inhibitory action of tannins on digestibility. The corelation was higher for the summer stage

between tannin estimates using Folin-Denis, vanillin-HCl and astrigency assays was found. This indicates that each woody species has a different proportion of the various tannin groups determined. Similar results have been reported by Burrit et al. (1987) working with woody species.

Folin-Denis assay

The species of genus Arbutus at both phenological stages had the highest total tannin concentrations (LSD 7.1, 19.4 respectively) (Table 1). High tannin concedntrations had also the species Ph.me., followed by the species Am.fr., Mo.al.cv., Gl.tr., and low the species Mo.al. and Co.ar. This was true for both seasons but especially for the spring phenological stage. A significant ($P \leq 0.05$) reduction in tannin concentration was observed (Table 1) from the spring to the summer phenological stage for almost all woody species studied except the species Ph.me. The decline in descending order was the following Mo.al.cv. (76%), Ro.ps. (70%), and Gl.tr. (69%), Mo.al (66.6%), Co.ar. (59%) Ar. un. (28%), Ar. an. (41.8%) and A. andr. (21.5%). The decline from the spring to the summer stage have been reported for other forage species (Hillis and Swain 1959). They have reported that the amounts of total phenolics constituents increased rapidly until the leaves reach maximum size and then decrease.

Vanillin-HCl assay

Variations of condenced tannin concentrations were less during spring than summer (LSD 1.8 vs 4.2) among species. During spring almost all species had twice as much tannin as that of the

(r = 0.556) than the spring one (r = 0.470). This was most probably a result of the different structure of the cellular constituents during the two phenological stages. Summer samples had a trend for higher astrigency effect. Furthermore, cellular structure has increased content of lignin, a factor which interferes with digestibility (Nastis 1982). Thus the higher correlation during the summer season can be attributed to the interference of tannin and lignin fractions. More detailed studies defining the extent and the role of each factor is needed so that to better understand their effect on digestibility. Further more, fractioning of tannin components into specific groups with different effect such as proanthocyanidins has to be investigated. We speculate that tannin effect may have similar effect on litter microflora and thus to soil productivity. However, this has to be further delineated.

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