Proanthocyanidins from *Hedysarum, Lotus* and *Onobrychis* spp. growing in Sardinia and Sicily and their antioxidant activity

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Keywords: proanthocyanidins, condensed tannins, quantification, antioxidant activity, quality

Introduction Proanthocyanidins (PA), or condensed tannins, are a class of natural polyphenolic compounds, occurring in numerous plant species, including a number of economically significant forage legumes. These compounds are polymers of flavan-3-ols, and typically contain from 2 to 20 units. Their biological significance is still being debated and, in recent years, a great deal of attention has been focused on their role in ruminant nutrition. Evidence has indicated that PA, in a moderate concentration (0.5-5% DM), may have considerable importance in protecting dietary proteins against microbial degradation in the rumen, and in preventing bloat. The antioxidant activity (AA) is also an important feature for animal well-being (Barry & McNabb, 1999). In order to study the PA content related to the antioxidant activity, samples of *Hedysarum, Lotus* and *Onobrychis* spp. from Mediterranean environments have been considered and investigated.

Materials and methods Forage plants analysed in this study were collected in hilly semi-arid areas of Sardinia and Sicily. The PA were extracted from the lyophilised plant samples with 30% aqueous acetone, and quantified by a spectrophotometric method following the butanol-HCl-Fe^{3+} assay (Porter *et al.*,1986). Delphinidin was used as standard, absorbance was read at 550 nm. After hydrolyses, a bidimensional TLC method was used to evaluate the monomers. The AA was performed by both FRAP (Ferric Reducing Antioxidant Power) and TEAC (Trolox Equivalent Antioxidant Capacity), as reported by Luximon-Ramma *et al.* (2002).

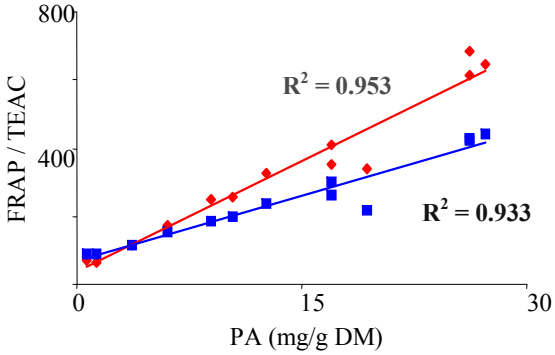

Figure 1 Correlation between PA and ♦ FRAP (µmol Fe(II) g^{-1} DM) and ■ TEAC (µmol Trolox g^{-1} DM)

Results The species under investigation, both from Sardinia and Sicily, showed a wide range of PA content. In *Lotus* spp. (*L.cytisoides, L. corniculatus, L. edulis, L.ornithopodioides*) PA content was lower (from 0.6 to 6.0 mg g^{-1} DM), compared to the different genotypes of *H. coronarium* (PA quantified from 8.9 and 17.0) and *H. spinosissimum* (19.4). Higher values were detected in *H. glomeratum* (26.2) and *O. viciifolia* (26.2-27.2). From the TLC investigation delphinidin and cyanidin were found as the major detected monomers, together with other compounds not further investigated. The AA values were 65-174 FRAP and 87-156 TEAC for *Lotus* spp., 252-406 FRAP and 183-262 TEAC for *H. coronarium* and *H. spinosissimum*, and 649-686 FRAP and 422-443 TEAC for *H. glomeratum* and *O. viciifolia*. The correlation between PA content and AA is shown in Figure 1.

Conclusions These results demonstrate that the PA content in the plant material under study was usually within the range considered beneficial in ruminant diet. The AA of the extracts, expressed as FRAP and TEAC, was strongly correlated with the PA content.

Acknowledgements The research was financed by MiPAF project ANFIT

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