**Tannin variation in tree fodder because of species, plant fraction and month**

**Application:** Tannin profiles of tree fodder vary between species, plant fraction and month. Therefore, it is recommended to perform tannin analysis before tree fodder harvest to ensure that the quality and quantity of tannins are appropriate to achieve the desired nutritional and environmental benefits.

**Introduction:** Tannins are one of the most abundant polyphenolic compounds in plants, with a multitude of chemically unique entities in nature (Besharati et al., 2022). Condensed tannins (CT) consist of flavan-3-ol subunits linked together to form oligomers and polymers (Besharati et al., 2022). They can reduce enteric methane emissions and improve nitrogen use efficiency and nutritional quality of animal-derived foods (Mueller-Harvey et al., 2019). On the other hand, they may reduce feed intake and digestibility or animal performance when fed in high amounts (dietary concentrations <50g CT/kg DM are recommended to achieve benefits) (Mueller-Harvey, 2006). However, it is difficult to establish a relationship between CT and their biological activities because of their chemical diversity and the lack of complete understanding of underlying mechanisms (Besharati et al., 2022). For example, procyanidins can be more effective against methanogens, while prodelphinidins have more phenolic groups and can for more H-bonds and higher degree of polymerisation and have improved antiparasitic effects (Mueller-Harvey et al., 2019). Given the increased interest in the use of tree fodder as ruminant feed in silvopastoral systems and the varied effect of tannins on animals, knowing the tannin profile of different tree fodders can promote the beneficial and prevent the damaging- impacts on livestock production. This study aimed to assess the tannin profile (quantitative and qualitative) in three tree fodder species (goat willow, oak, and maple) and their plant fractions (leaves and twigs) throughout the grazing season (June-September).

**Material and methods:** Leaf and twig samples were collected from three species (goat willow, *Salix caprea*; oak, *Quercus robur*; field maple, *Acer campestre*) from Elm Farm, an 85 ha organic livestock farm in West Berkshire, UK, with soil types varying from heavy clay loam to sandy loam. Five trees per species (n=15) were sampled monthly from June - September 2017. The concentration of CT was determined using a Butanol−HCl assay. Qualitative analysis on the molar percentages of flavan-3-ol subunits, mean degree of polymerization (mDP), procyanidins (PC) and prodelphinidins (PD) proportions, and *cis*- versus *trans*- flavan-3-ols subunits was conducted using *in situ* thiolysis assay (Natalello et al. 2020). In-vitro true dry matter digestibility (DMD) was determined using the ANKOM Daisy II system. All analyses were conducted in triplicate. Data were analysed using linear mixed models in Minitab Statistical Software 20.2, using species, plant fractions, month, and their interactions as fixed factors, and tree ID (nested within species) as random factor. Tukey's Honestly Significant Difference test was conducted for pairwise comparisons when the effect of a fixed factor was statistically significant (P<0.05).

**Results:** Dry matter content of fodder was highest for maple (484 g/kg fresh), lowest for goat willow (445 g/kg fresh) and intermediate for oak (464 g/kg fresh) (P<0.001, SE=7.8). Dry matter digestibility was highest for goat willow (559 g/kg DM), followed by oak (497 g/kg DM) and maple (525 g/kg DM) (P<0.001, SE=7.8). Total CT and *trans-* flavan-3-ols contents were highest for goat willow (69.5 g/kg DM; 71.7% of CT), intermediate for oak (35.7 g/kg DM; 64.1% of CT) and lowest for maple (24.1 g/kg DM; 13.2% of CT), while the opposite was observed for *cis* flavan-3-ols when expressed as % of CT (28.3; P<0.001; SE=2.9). Procyanidins were highest in maple (73.4% of CT), lowest in oak (45.2% of CT) and intermediate in goat willow (54.7% of CT), while the opposite was observed for prodelphinidins when expressed as % of CT (26.6; P<0.001, SE=4.8). When compared with twigs, leaves had higher DMD (639 vs 415 g/kg DM, SE=7.0, P<0.001), and total CT concentrations (47.6 vs 38.6 g/kg DM, SE=3.0, P<0.001); and lower mDP (3.94 vs 4.47, SE=0.3, P=0.002). There was a variation (3.62-5.52, SE=0.4, P<0.001), but month did not affect other CT parameters.

**Conclusion:** The present study demonstrates that there is a significant effect of species, plant fractions and month on DMD and tannin profile of tree fodder. The differing effects of tannin types on animal metabolism and the high variation between species, tannin contents and profiles should therefore be accounted for when introducing tree fodder to animal nutrition, to achieve the desirable effects by minimizing the risk of anti-nutritional impacts.

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