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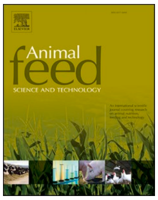

# Tannin variation in tree fodder from temperate climates and implications for methane emissions from enteric fermentation

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## ABSTRACT

A field trial evaluated the effects of tree species, plant fractions, and season on the dry matter degradability (**DMd**) and tannin profiles of three tree species (*Salix caprea,* goat willow (**GW**); *Quercus* robur, common oak; *Acer campestre.*, maple). Leaf and twig samples were collected monthly from five trees per species between June and September in Berkshire, UK. Total condensed tannins (**CTs**) concentrations were determined using a Butanol−HCl assay and tannin profiling (mean degree of polymerization (**mDP**), and concentrations of procyanidins (**PC**), prodelphinidins (**PD**), and *cis*- and *trans*- flavan-3-ols) were measured via *in situ* thiolysis assay. *In vitro* total DMd was determined using the ANKOM Daisy II system. Data were analysed using linear mixed models with tree species, plant fractions, month, and their interactions as fixed factors, and tree ID (nested within species) as a random factor. Goat willow had the highest ($P < 0.05$) DMd, total CTs concentrations, mDP, and *trans* flavan-3-ols. Oak had the highest ($P < 0.001$) PD concentrations. Maple had the highest final DM, soluble losses, and concentrations of PC, and *cis* flavan-3-ols. Across all tree species, leaves had a higher ($P < 0.001$) DMd, soluble losses, and total CTs concentrations, and lower DM ($P < 0.001$) and mDP ($P = 0.043$) concentrations than twigs. Soluble losses and DMd increased ($P < 0.001$) with season, from June to September, while the highest ($P = 0.001$) DM content was observed in August. Tannin profiles did not vary with seasons, except for the highest ($P < 0.001$) mDP concentrations in June compared with other months. The impacts of high-PC (96 % in CTs; HPC) and high-PD (73 % in CTs; HPD) GW on total gas and methane (**$CH_4$**) production were assessed *in vitro*. Experimental diets were 100 % grass silage (GS, control), HPC (80:20 grass silage:HPC GW), HPD (80:20 grass

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*Abbreviations:* $CH_4$,, methane; CTs, Condensed tannins; DM, dry matter; DMd, dry matter degradability; GC, gas chromatography; GHG, greenhouse gas emissions; GW, goat willow; HPC, high-procyanidins; HPD, high-prodelphinidins; IVDMD, in vitro dry matter degradability; MDP, mean degree of polymerisation; N, nitrogen; PCs, Procyanidins; PDs, Prodelphinidins; PEG, polyethylene glycol; RoP, extent of degradation in rumen proper at given passage rate of 0.04 h and 0.025 h; VFA, volatile fatty acids.

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silage: HPD GW), plus two diets with the addition of polyethylene glycol (**PEG**) in the HPC and HPD to neutralise tannins. Diets were incubated for 72 h in rumen fluid: medium at 1:9 v/v in triplicate. Gas pressure (psi transducer) and $CH_4$ concentrations (gas chromatography) were measured throughout. Volatile fatty acids (**VFA**) concentrations and DMd were assessed at 72 h. Data were analysed by linear mixed models using dietary treatment as a fixed factor and batch run (1−3) as a random factor. HPC, HPD, HPC+PEG, and HPD+PEG diets had lower ($P < 0.001$) DMd and 72 h production of cumulative gas and $CH_4$ than Grass silage (**GS**). Grass silage produced more ($P < 0.001$) total VFA and acetic acid concentrations than the four GW diets and less ($P < 0.001$) butyric acid than HPD. This study concludes that the inclusion of GW reduced *in vitro* $CH_4$ production, partly due to its tannin content, with PC showing an improved potential. Species and fraction-specific differences in tannin profiles influenced the nutritive value and $CH_4$ mitigation potential of tree fodder, and these need to be accounted for when tree fodder is introduced into animal diets.

## 1. Introduction

Introducing tree fodder in grazing animal diets has received attention recently due to its potential multiple benefits (Domiciano et al., 2020). Planting trees on farmland, particularly in temperate climates, offers a wide range of environmental advantages, such as improving air and water quality (Vandermeulen et al., 2018; Domiciano et al., 2020); e.g., hedgerows in temperate regions can mitigate undesirable odours around livestock farms, while trees help reduce wind and water erosion, promoting soil stabilisation (Coussement et al., 2018). In temperate climates, trees also significantly influence carbon sequestration both above and below ground (Adewopo et al., 2014; Domiciano et al., 2020) and improve soil quality through nutrient cycling. Trees can fixate atmospheric nitrogen used by the plant to produce protein, while it can be diverted to the nearby pasture plants (Gardiner et al., 2013; Coussement et al., 2018). Beyond these environmental benefits, trees in temperate farmlands promote biodiversity by providing habitats for flora and fauna, and enhance the landscape's aesthetic value (Domiciano et al., 2020). Agroforestry systems, called silvopastoral when grazing animals are involved, are multifunctional land-use systems that associate livestock with shrubs, trees and pasture, improving animal production, health, welfare and nutrition (Etienne, 1996; Kohari et al., 2007; Jose, 2009; Masters et al., 2023). The interest in such modern silvopastoral systems has increased, and trees can provide browsing, which may be used as an extra feed resource for ruminants from trees, whilst also potentially producing firewood, construction materials, fruits and/or nuts, as well as providing shade and shelter for livestock against adverse weather conditions (Franzel et al., 2014; Smith et al., 2014). Tree fodder has an advantage over other feed resources because it grows naturally and is produced locally on farms and in nearby areas. They are contributing to improved resiliency of silvopasture-based livestock production systems (Franzel et al., 2014). Many trees remain green during most of the dry season when pasture is dry or of poor nutritional quality (Moore et al., 2003; Franzel et al., 2014).

Tree fodder provides a cheap and readily available source of proteins for grazing animals as they contain between 10 % and 30 % crude protein on a dry matter basis (Mahieu et al., 2021). Tree fodder is also rich in other essential nutrients, including soluble carbohydrates, minerals, and vitamins (Mtui et al., 2008). However, more information is needed on how to sustainably introduce tree fodder in more intensive ruminant production systems, particularly in temperate climates, where seasonal variations in tree growth and nutrient availability may play a role (Vandermeulen et al., 2018). Particular interest in tree fodder's nutritional properties, both for grazing and indoor-fed animals, is their concentrations of condensed tannins (**CTs**). Feeding livestock with CTs containing tree fodder can enhance nitrogen (**N**) recycling in the pasture by a shift from excretion via urine to faeces (Waghorn et al., 1987), in which N is less volatile, leading to balance in the rumen function by decreasing ruminal N degradation and then reducing urinary N losses and $N_2O$ emissions (De Klein and Eckard, 2008). CTs can also reduce enteric methane (**$CH_4$**) emissions, protect grazing animals from frothy bloat (accumulation of gas in the rumen and reticulum), and improve the nutritional quality of animal-derived foods (Mueller-Harvey et al., 2019a; Besharati et al., 2022). The beneficial or adverse effects of CTs on ruminant nutrition depend on their amount, type, chemical structure, and feed composition (Piluzza et al., 2014; Besharati et al., 2022).

In addition to the factors above, the effect of CTs on animal production and health may vary due to the differences in procyanidins (**PC**) to prodelphinidins (**PD**) ratio (PC:PD), a topic that is poorly assessed in the literature as most studies investigate the effect of total CTs rather than the effect of tannin profile. Most studies have focused on total CT content rather than the tannin profile, which likely contributes to the inconsistent results on $CH_4$ emissions observed both *in vitro* and *in vivo*. Variations in CT concentration and PC:PD ratios are influenced by factors such as soil composition, light intensity, and temperature (Besharati et al., 2022). Research has shown that tannin-rich plants reduce $CH_4$ production due to their antimicrobial properties (Theodoridou et al., 2011; Morgavi et al., 2012; Jayanegara et al., 2015; Molina-Botero et al., 2019). *In vitro* studies reported a 22.3–36.7 % reduction in methanogen populations when CTs were added at 1.0 mg/mL. Additionally, plant extracts from *Acacia cornigera* and a mixture of tanniferous plants reduced $CH_4$ by 14 % and 7 %, respectively, after 24 h of incubation (Rodríguez et al. (2011). *In vivo,* feeding lambs forages such as *Leucaena leucocephala*, *Styzolobium terrimum*, and *Mimosa caesalpiniaefolia* at different inclusion rates showed that *Leucaena leucocephala* had the most significant $CH_4$ reduction (~25 %) compared to other forages (Moreira et al., 2013).

Methane is a potent greenhouse gas emissions (**GHG**) with a 28-fold more significant global warming potential than $CO_2$ (Gerber et al., 2013; Pachauri et al., 2014). Approximately 15 % of all anthropogenic GHG emissions are produced by livestock (Gerber et al., 2013). Therefore, reducing $CH_4$ emissions from ruminant livestock is an essential goal for reducing the climate impact of agriculture (Sari et al., 2022). Methanogenesis, driven by methanogenic archaea, plays a key role in ruminant digestion, and mitigation strategies must avoid impairing animal performance (Beauchemin et al., 2020). Redirecting hydrogen from methanogenesis to beneficial fermentation products could reduce $CH_4$ emissions and improve productivity, but this requires further investigation (Beauchemin et al., 2020).

To fully exploit the potential of tree fodder in temperate climates for $CH_4$ mitigation in ruminant production systems, it is necessary to understand the factors affecting their tannin profiles [PC, PD; as well as their stereochemistry (*cis*, *trans*)] and their mean degree of polymerisation (**mDP**). These structural characteristics likely influence the $CH_4$ mitigation capacity of tree fodder, particularly in temperate climates where these factors may be subject to seasonal and environmental variation. This study, therefore, aimed to investigate (i) the effect of three tree species (*Salix caprea*, GW; *Quercus robur*, common oak; *Acer campestre*, maple), plant fractions (leaves and twigs), month (June, July, August, and September), and their interactions on total CTs and their profile (PC, PD, *cis*, *trans*, mDP), via a field trial, and (ii) the effect of CTs profiles (high concentrations of PC, HPC; high concentrations of PD, HPD) on total gas and $CH_4$ production, via an *in vitro* gas production system.

## 2. Materials and methods

### 2.1. Fodder sampling and analysis

#### 2.1.1. Collection of tree fodder species

Fresh leaf and twig samples were collected from three tree species: oak (*Quercus robur*), maple (*Acer camprestre*), and goat willow [**GW** (*Salix caprea*)] and then dried post-collection from Elm Farm, an 85 ha organic livestock farm in West Berkshire (51° 23′ 14.19″ N; 1° 24′ 08.34″ W; altitude 90–130 above sea level), with soil types varying from heavy clay loam to sandy loam (Eutric Luvic Planosols). For each species, the same five trees were sampled monthly from June to September 2017. The sampled trees were spread throughout the farm and either in mixed species boundary hedges or individual trees in-field. Sampled trees of each species were at least 500 m apart. Leaf and twig samples were obtained by selecting branches with a maximum diameter of 10 mm around the tree crown and cutting using secateurs from four orientations (North, East, South, and West), to provide a total of at least 1.2 kg cut from each tree every month. The leaf and twig fractions were then divided by stripping the leaves and petioles from the cut branches using gloves into a paper bag, to obtain a sample of at least 600 g (fresh weight) of each. The samples were transported in large plastic bags back to the on-farm laboratory and processed immediately.

#### 2.1.2. Condensed tannins analysis

Condensed tannins (CTs) were determined using the modified Butanol−HCl assay according to the method developed by (Grabber et al., 2013) with some modifications. Briefly, the reagent was prepared with 150 mg of ammonium iron (III) sulfate, 3.3 mL of water, 5.0 mL of 12 M HCl, 42 mL of n-butanol, and 50 mL of acetone. A volume of 10 mL of the daily fresh reagent was mixed with 10 mg of dried tree fodder sample. After 1 h at room temperature in darkness, tubes were incubated in a heating/stirring module (Reacti-Therm III TS-18823; Thermo Scientific; Waltham, Massachusetts, United States) at 70ºC for 2.5 h in the dark while being stirred by a small magnetic bar. After cooling to room temperature, reaction mixtures were scanned with a spectrophotometer from 450 to 650 nm, and the maximal absorption of the anthocyanidin peak was recorded. Condensed tannins were quantified using a purified tannin fraction from sainfoin (*Onobrychis viciifolia*) as a secondary standard.

#### 2.1.3. Condensed tannins – In situ thiolysis

Total CTs concentrations, molar percentages of flavan-3-ol subunits, mean degree of polymerization (**mDP**), procyanidins (**PCs**), and prodelphinidins (**PDs**) proportions, and *cis*- versus *trans*- flavanols subunits were determined in triplicates using *in situ* thiolysis assay according to the method of Gea et al. (2011) with a few minor modifications described by Natalello et al. (2020).

**Table 1**
Nutrient composition and energy content of dried grass silage, goat willow high in procyanidin (HPC), goat willow with in prodelphinidin (HPD), and the two experimental diets (including HPC and HPD).

| | Ingredients[a] | | | Diets | |
|---|---|---|---|---|---|
| Feed composition[b] | Grass silage | Goat willow (HPC) | Goat willow (HPD) | HPC Diet | HPD Diet |
| DM (g/kg) | 930 | 940 | 931 | 932 | 931 |
| Ash (g/kg) | 156 | 50 | 49 | 135 | 134 |
| CP (g/kg DM) | 167 | 145 | 139 | 163 | 161 |
| Total Oil (g/kg DM) | 47.4 | 31.8 | 31.6 | 44.3 | 44.2 |
| Ether Extract (g/kg DM) | 42.5 | 19.9 | 20.4 | 38.0 | 38.1 |
| NDF (g/kg DM) | 472 | 346 | 337 | 447 | 445 |
| ADF (g/kg DM) | 339 | 478 | 388 | 367 | 345 |
| GE (MJ/kg DM) | 17.3 | 21.1 | 22.5 | 18.1 | 18.4 |

[a] HPC Diet = 800 g/kg grass silage and 200 g/kg goat willow HPC on DM basis; HPD Diet = 800 g/kg grass silage and 200 g/kg goat willow HPD on DM basis

[b] DM = Dry matter; CP = Crude protein; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; GE = Gross energy.

## 2.2. *In vitro gas production*

All animal procedures were performed under the authority of the UK Animals (Scientific Procedures) Act, 1986, after approval by the local animal welfare and ethical review body and local ethical clearance (DAS/PGR2023NFS02/03RF).

### 2.2.1. *In vitro experiment*

The *in vitro* gas production experiment was conducted using a method adapted from (Theodorou et al., 1994; Mauricio et al., 1999) and (Sinclair et al., 2005). For each incubation time, each sample (0.2 g) of the two substrates < 2 mm (GW with high PC and GW with high PD) with and without PEG was weighed and added to 0.8 g dried, milled (< 2 mm) silage of pure perennial ryegrass substrate in Wheaton flasks. In the experimental treatments containing PEG, the quantity used 2.3 g/l of phosphate:carbonate buffer solution (McSweeney et al., 1999). Grass silage-only flasks and negative control flasks (no substrate) were also prepared. The nutrient composition of the experimental diet ingredients and total diets are presented in Table 1. Each treatment/control was incubated in triplicates at 39 °C in each flask that contained 90 mL buffer (Mauricio et al., 1999) and 10 mL rumen fluid (obtained pre-feeding from a dry dairy cow maintained on a high-forage (grass hay/silage) and strained through two layers of cheesecloth). At 2, 4, 6, 8, 10, 12, 24, 32, 48, and 72 h of incubation, the gas pressure was recorded using a headspace pressure transducer (Bailey and Mackey Ltd., Birmingham) and recorder (psi; Tracker 200; Data Track Process Instruments, UK), and a sample of gas was collected for $CH_4$ analysis. At 72 h, flask lids were removed, and the pH of the contents was recorded before a subsample of fluid was taken and stored at −20 °C for volatile fatty acids (**VFA**) analysis. Flask contents were filtered through pre-weighed sintered glass crucibles, and the residue was oven-dried at 100 °C for 4 h before reweighing to obtain dry residue weight. Each batch culture run for each treatment was run on three occasions using rumen fluid from the same donor animal. Gas pressure readings (psi) were used to calculate the gas volume using an equation that takes into account diffusion of gas into the liquid phase (Mauricio et al., 1999). Gas volume (mL) was adjusted to take into account blank volume (negative control) at each time point and expressed as mL/g substrate. Methane concentration of gas samples taken was measured using gas chromatography (GC) following manual injection of gas samples. *In vitro* dry matter degradability (IV**DMD**, after 72 h) was calculated using the total weight of substrate added at time 0 and the dry weight of residue after 72 h. pH values were converted to hydrogen ion concentrations ([H+]) prior to statistical analysis, and then means from the model were log converted to calculate pH. Gas and $CH_4$ volumes were fitted to previously published model of gas production (France et al., 1993).

### 2.2.2. *Methane production analysis*

Methane was analysed by gas chromatography (**GC**). Prior to measurement, a five-point (25,000, 50,000, 75,000, and 100,000 ppm) standard curve was conducted on the GC (Bruker 450 GC, Bruker, Germany) fitted with a port valve and plumbed into the injector. Methane concentrations obtained using the GC were applied to the gas volumes to calculate CH4 volume (mL/g substrate). Full column and GC conditions are reported in Muñoz-González et al. (2012). Gas components were separated on a methane-packed Poropak N column (1.2 m, 2 mm internal diameter, Varian Inc., Walnut Creek, CA) and CH4 was detected using a flame ionisation detector.

### 2.2.3. *Volatile fatty acids analysis*

Subsamples of 72 h incubation medium were thawed, and 1.2 mL thawed sample was added to 0.3 mL internal standard (25 mM 2-ethylbutyric acid in 25 % w/v metaphosphoric acid) and allowed to stand for 30 min. Samples were then centrifuged at 1570 g for 10 min at 4 °C, before 0.5 mL supernatant was transferred to a GC vial containing 0.8 mL distilled water. A series of standards (containing acetic, propionic, butyric, vaccenic, isobutyric, isovaleric and caproic acids) were prepared in the same way. Volatile fatty acids in 1.5 μl standards and samples were separated using GC (Agilent 7890B) equipped with a flame ionisation detector and a 30 m column (30 m, 0.25 mm internal diameter, 10 m guard; Stabilwax-DA). The oven temperature started at 70 °C and increased by 24 °C per min to a temperature of 190 °C. Then, it increased by 90 °C/min to 235 °C and held for 0.5 min. The injector and detector temperatures were set at 220 °C and 250 °C, respectively, and split injection was employed (16:1). Hydrogen was used as a carrier gas, at a constant flow of 2.0 mL/min. Results for each VFA were recorded on a mM basis.

## 2.3. *Statistical analysis*

### 2.3.1. *Field trial*

Data were analysed using linear mixed models in SPSS (version 29.0, IBM, Armonk, NY, USA), using species, plant fractions, month, and their interactions as fixed factors and tree ID (nested within species) as a random factor. Month and plant fractions within tree ID were also considered as repeated effects. Repeated effects used the covariance structure (autoregressive, heterogeneous autoregressive, compound symmetry, heterogeneous compound symmetry, ante-dependence, or unstructured) giving the best fit based on the lowest Bayesian information criterion (BIC) value and the homogeneity of each variable. The normal distribution of the residuals was assessed visually. Normality was violated for DMd, cis, trans, and mDP and data were log-transformed prior to analysis by the linear mixed models. All other variables were analysed untransformed. Where the effect of the fixed factors or their interactions were statistically significant ($P < 0.05$), Fisher's Least Significant Difference Test ($P < 0.05$) was used for the pairwise comparisons of the means.

### 2.3.2. *In vitro gas production system*

Gas and $CH_4$ model parameters, VFA concentrations, pH, and IVDMD were analysed using linear mixed models in Minitab (Minitab Statistical Software version 20.2), using treatment (control, high-PC, high-PD, high-PC plus PEG, high-PD plus PEG), as a fixed factor, and batch run as a random factor. Normal distribution of the residuals was assessed visually and there were no deviations from normality, and all variables were analysed untransformed. Tukey's Honestly Significant Difference test was conducted for multiple comparisons between treatment means, and the statistical significance of the treatment effect was considered at ($P < 0.05$).

## 3. Results

### 3.1. Field trial

### 3.1.1. Effect of tree species, plant fractions and month on tannin profile

Tree species influenced final DM ($P = 0.021$), DMd ($P < 0.001$), soluble losses ($P < 0.001$), total CTs concentrations ($P < 0.001$), mDP ($P = 0.017$), PC ($P < 0.001$), PD ($P < 0.001$), *cis* ($P < 0.001$) and *trans* ($P < 0.001$) (Table 2). DM content and *cis* were highest in maple, had intermediate values in oak (27 g/kg fresh and 59.2 % CTs lower compared with maple), and lowest in goat willow (by 45 and 18 g/kg fresh, and 66.8 %, 7.6 % CTs compared with maple and oak, respectively). DMd was highest in goat willow (GW), had intermediate values in maple (34 g/kg DM lower in maple), and lowest in oak (by 62 and 28 g/kg DM compared with GW and maple, respectively). Soluble losses were lowest in GW (by 88 and 103, compared with oak and maple, respectively). Total CTs content and *trans* were highest in GW, had intermediate values in oak (by 14 g/kg DM and 7.6 % CTs, lower compared with GW), and lowest in maple (by 45.6 and 11.6 g/kg DM, and 66.8 and 59.2 % CTs compared with GW and oak, respectively). PC content was highest in maple, had intermediate values in GW (by 43.8 % CTs lower compared with maple), and lowest in oak (by 53.3 and 9.5 % CTs compared with maple and GW, respectively), while the opposite was observed for PD content. Furthermore, mDP was the highest in maple, had intermediate values in GW (0.18 lower compared with maple), and lowest in oak (1.18 and 1.35 lower compared with GW and maple).

The plant fractions had a significant effect on final DM ($P < 0.001$), DMd ($P < 0.001$), soluble losses ($P < 0.001$), total CTs concentrations ($P < 0.001$) and mDP ($P = 0.043$) (Table 2). When compared with twigs, leaves had higher DMd (+224 g), soluble losses (+85), and total CTs (+9 g/kg DM), but lower final DM (-102 g) and mDP (-0.15).

Final DM ($P = 0.001$), DMd ($P < 0.001$), soluble losses ($P < 0.001$), and mDP ($P < 0.001$) varied with month (Table 2). Soluble losses were the highest in September and DMd was higher in August and September compared to June and July. Final DM was the highest in August while there was no difference in final DM between other months. In June, mDP was the highest compared to July and August.

### 3.1.2. Effect of the interactions between species, plant fractions, and month on tannin profile

The interaction between species and plant fractions had a significant effect on Final DM ($P < 0.001$), DMd ($P < 0.001$), total CTs ($P = 0.004$), PC ($P < 0.001$), PD ($P < 0.001$), *cis* ($P < 0.001$), *trans* ($P < 0.001$) and mDP ($P < 0.001$) (Table 3). GW, oak, and maple-twigs had higher final DM than leaves (+77, +99, and +129 g/kg fresh, respectively). Maple leaves had higher final DM than GW and oat ones (+20 and +13 g/kg fresh, respectively). Leaves had higher DMd than twigs for GW, oak, and maple (+206, +177, +289 g/kg DM, respectively). GW and maple had higher leaves-DMd compared with oak (+76 and +83 g/kg DM, respectively), while for twigs DMd was higher for GW compared with oak and maple (+47 and +76 g/kg DM, respectively). Total CTs in leaves were higher compared with twigs for GW (+14 g/kg DM) and oat (+9.5 g/kg DM). Total CTs were higher for GW than oak and maple (+36.3 and +51 g/kg DM, respectively), while oak had higher than maple (+14.7 g/kg DM). Total CTs in twigs were also higher for GW than oak and maple (+31.8 and +40.2 g/kg DM, respectively). PC were higher in oak leaves than in twigs (+20.9 % total CTs). Additionally, maple had higher PC in leaves and twigs compared with GW (+42.6 and +44.9 % total CTs for leaves and twigs, respectively) and oak (+43.2 and 63.3 % total CTs for leaves and twigs, respectively). PD were higher in oak twigs than leaves (+20.9 % total CTs). GW and oak had higher leaves-PD (+42.6 and +43.2 % total CTs, respectively), and twigs-PD (+44.9 and +63.3 % total CTs, respectively). GW had higher *cis* in twigs than leaves (+15.3 % total CTs), while oak had higher in leaves than twigs (+13.4 % total CTs). Maple had higher *cis* in leaves compared with GW (+75.1 % total CTs) and oak (+53.2 % total CTs), while oak had higher compared with GW (+21.9 % total CTs). In twigs, *cis* was higher for maple compared with GW (+58.5 % total CTs) and oak (+65.3 % total CTs). In GW *trans* were higher in leaves than twigs (+15.3 % total CTs), while the opposite was found for oak (+13.4 % total CTs) and maple (+1.34 % total CTs). Compared with maple, GW and oak had higher *trans* in leaves (+75.1 and +53.2 % total CTs, respectively) and twigs (+58.5 and +65.3 % total CTs, respectively). Oak twigs had higher mDP than oak leaves (+1.28), while maple twigs had higher mDP than maple leaves (+1.16). GW and maple leaves had higher mDP than oak leaves (+1.66 and 2.57 for GW and maple, respectively).

The interaction between species and month had a significant effect on soluble losses ($P = 0.003$), and *trans* ($P = 0.011$) (Table 4). Soluble losses were higher in GW for August and September compared with June and July, in oak for July-September than June, and in maple in September compared with June-August. Furthermore, maple had the highest soluble losses in June compared to GW (+105 g/kg DM) and oat (+60 g/kg DM) and oat compared with GW (+45 g/kg DM). Compared with GW, both oak and maple had higher soluble losses in July (+122 and +111 g/kg DM, respectively), August (+100 and +85 g/kg DM, respectively), and September (+83 and +110 g/kg DM, respectively). Compared maple, both GW and oak had higher *trans* in June (+63.1 and +55.3 % total CTs, respectively), July (+68.4 and +58.9 % total CTs, respectively), August (+67.9 and +64.5 % total CTs, respectively), and September (+67.9 and +58.5 % total CTs, respectively). Within maple, *trans* in June were higher than July-September.

The interaction between plant fractions and month had a significant effect on mDP ($P = 0.007$) (Table 5). mDP was higher (+0.5) in August twigs than in August leaves. June leaves had higher mDP compared with July (+0.8), August (+1.17), and September (+0.75). July and September also had higher mDP compared with August (+0.37 and +0.42, respectively).

**Table 2**

Means and standard error (SE) for the effect of species (goat willow, oak, maple), plant fractions (leaves, twigs) and month (June-September) on dry matter (DM) content and degradability (DMd), and condensed tannins (CTs) content and profiles.

| | Species | | | | | Plant fractions | | | | Month | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameters[a] | Goat Willow n = 40 | Oak n = 40 | Maple n = 40 | SE | *P*-value[b] | Leaves n = 60 | Twigs n = 60 | SE | *P*-value[b] | June n = 30 | July n = 30 | Aug n = 30 | Sept n = 30 | SE | *P*-value[b] |
| DM (g/kg fresh) | 466$^{b}$ | 484$^{ab}$ | 511$^{a}$ | 9.87 | **0.021** | 436 | 538 | 6.1 | **< 0.001** | 471$^{b}$ | 482$^{b}$ | 515$^{a}$ | 481$^{b}$ | 8.4 | **0.001** |
| **Degradability** | | | | | | | | | | | | | | | |
| DMd (g/kg DM) | 559$^{a}$ | 497$^{b}$ | 525$^{b}$ | 9.7 | **< 0.001** | 639 | 415 | 6.6 | **< 0.001** | 499$^{b}$ | 511$^{b}$ | 543$^{a}$ | 555$^{a}$ | 7.7 | **< 0.001** |
| Soluble losses (g/kg DM) | 306$^{b}$ | 394$^{b}$ | 409$^{a}$ | 5.6 | **< 0.001** | 412 | 327 | 4.2 | **< 0.001** | 331$^{d}$ | 360$^{c}$ | 379$^{b}$ | 408$^{a}$ | 6.2 | **< 0.001** |
| **Condensed tannins (CTs)** | | | | | | | | | | | | | | | |
| Total CTs (g/kg DM) | 69.7$^{a}$ | 35.7$^{b}$ | 24.1$^{b}$ | 4.23 | **< 0.001** | 47.6 | 38.7 | 2.52 | **< 0.001** | 42.4 | 42.5 | 42.8 | 44.7 | 2.77 | 0.691 |
| Procyanidins (% CT) | 54.7$^{b}$ | 45.2$^{b}$ | 98.5$^{a}$ | 7.59 | **< 0.001** | 70.3 | 62.0 | 4.43 | **< 0.001** | 66.4 | 65.7 | 66.6 | 65.9 | 4.42 | 0.751 |
| Prodelphinidins (% CT) | 45.3$^{a}$ | 54.8$^{a}$ | 1.48$^{b}$ | 7.594 | **< 0.001** | 29.7 | 38.0 | 4.43 | **< 0.001** | 33.6 | 34.3 | 33.4 | 34.1 | 4.42 | 0.751 |
| *cis* (% CTs) | 28.3$^{b}$ | 35.9$^{b}$ | 95.1$^{a}$ | 3.74 | **< 0.001** | 53.0 | 53.2 | 2.26 | ***0.052*** | 54.4 | 53.9 | 50.5 | 53.8 | 2.39 | 0.162 |
| *trans* (% CTs) | 71.7$^{a}$ | 64.1$^{a}$ | 4.87$^{b}$ | 3.739 | **< 0.001** | 47.0 | 46.8 | 2.26 | 0.246 | 45.6 | 46.1 | 49.5 | 46.2 | 2.39 | ***0.087*** |
| mDP$^{2}$ | 4.81$^{a}$ | 3.63$^{b}$ | 4.98$^{a}$ | 0.343 | **0.017** | 4.40 | 4.55 | 0.212 | **0.043** | 4.89$^{a}$ | 4.33$^{b}$ | 4.16$^{b}$ | 4.52$^{ab}$ | 0.224 | **< 0.001** |

[a] DM = Dry matter; DMd = Dry matter degradability; mDP = mean degree of polymerisation.

[b] Significances were declared at $P < 0.05$. Significant differences between dietary treatments within variable are indicated with different superscript letters according to Fisher's Least Significant Difference test ($P < 0.05$).

**Table 3**

Means and standard error (SE) for the effect of the interaction between species (goat willow, oak, maple) and plant fractions (leaves, twigs) on dry matter (DM) content and degradability (DMd), and condensed tannins (CTs) content and profiles.

| Parameters[a] | Goat Willow | | Oak | | Maple | | SE | *P*-value[b] |
|---|---|---|---|---|---|---|---|---|
| | Leaves n = 20 | Twigs n = 20 | Leaves n = 20 | Twigs n = 20 | Leaves n = 20 | Twigs n = 20 | | |
| DM (g/kg fresh) | $427^{B}$ | $504^{b,A}$ | $434^{B}$ | $533^{b,A}$ | $447^{B}$ | $576^{a,A}$ | 10.6 | **< 0.001** |
| **Degradability** | | | | | | | | |
| DMd (g/kg DM) | $662^{a,A}$ | $456^{a,B}$ | $586^{b,A}$ | $409^{b,B}$ | $669^{a,A}$ | $380^{c,B}$ | 11.4 | **< 0.001** |
| Soluble losses (g/kg DM) | 340 | 272 | 442 | 345 | 454 | 363 | 7.2 | ***0.085*** |
| **Condensed tannins (CTs)** | | | | | | | | |
| Total CTs (g/kg DM) | $76.7^{a,A}$ | $62.7^{a,B}$ | $40.4^{b,A}$ | $30.9^{b,B}$ | $25.7^{c}$ | $22.5^{b}$ | 4.36 | **0.004** |
| Procyanidins (% CTs) | $56.3^{b}$ | $53.2^{b}$ | $55.7^{b,A}$ | $34.8^{b,B}$ | $98.9^{a}$ | $98.1^{a}$ | 7.67 | **< 0.001** |
| Prodelphinidins (% CTs) | $43.7^{a}$ | $46.8^{a}$ | $44.3^{a,B}$ | $65.2^{a,A}$ | $1.09^{b}$ | $1.87^{b}$ | 7.67 | **< 0.001** |
| *cis* (% CTs) | $20.7^{c,B}$ | $36.0^{b,A}$ | $42.6^{b,A}$ | $29.2^{b,B}$ | $95.8^{a}$ | $94.5^{a}$ | 3.92 | **< 0.001** |
| *trans* (% CTs) | $79.3^{a,A}$ | $64.0^{a,B}$ | $57.4^{a,B}$ | $70.8^{a,A}$ | $4.20^{b,B}$ | $5.54^{b,A}$ | 3.920 | **0.004** |
| mDP | $4.65^{a}$ | 4.98 | $2.99^{b,B}$ | $4.27^{A}$ | $5.56^{a,A}$ | $4.40^{B}$ | 0.275 | **< 0.001** |

[a] DM = Dry matter; DMd = Dry matter degradability; mDP = mean degree of polymerisation.

[b] Significances were declared at $P < 0.05$. Significant differences between dietary treatments within plant fractions are indicated with different lowercase superscript letters and between plant fractions within dietary treatment are indicated with different uppercase superscript letters according to Fisher's Least Significant Difference test ($P < 0.05$).

## *3.2. In vitro gas production*

### *3.2.1. Effect of tannin profile in goat willow (GW) on in vitro dry matter degradability (IVDMD) and rumen fermentation parameter*

Goat willow had a significant effect on IVDMD ($P < 0.001$), RoP at 0.03 h ($P < 0.001$), RoP at 0.025 ($P < 0.001$), acetic acid ($P < 0.001$), propionic acid ($P < 0.001$), butyric acid ($P < 0.001$), total VFA concentrations ($P < 0.001$), acetic acid (% VFA) ($P < 0.01$), propionic acid (% VFA) ($P < 0.001$), butyric acid (% VFA) ($P < 0.001$), caproic acid (% VFA) ($P < 0.05$), and acetic: propionic ratio ($P < 0.001$).

When compared with the four treatments (HPC, HPD, HPC-PEG, HPD-PEG), Control had higher IVDMD (+7.1, +7.7, +5.7, +7.4 g/100 g, respectively), and concentrations of acetic acid (+2.5, +2.5, +2.4, +5.1 mM; and (+1.7, +1.7, +0.9, +0.3 % VFA; respectively). Both RoPs (at 0.03 and 0.25 h) were highest in HPC-PEG, lowest in HPD and HPD-PEG, and had intermediate values in Control and HPC. When compared with Control, concentrations of propionic acid were lower for HPC (-0.5 mM) and HPD (-0.5 mM) as well as HPC-PEG (-1.8 mM) and HPD-PEG (-2.9 mM). The PEG containing treatments (HPC-PEG, HPD-PEG) also had lower concentrations of propionic acid compared to their corresponding treatments without PEG (HPC, −1.3 mM; HPD, −2.4 mM). However, when propionic acid was expressed as % VFA, there was no difference between Control, HPC, and HPC, whilst there was a reduction in the concentrations of HPC-PEG (-1.5 %VFA vs HPC, and −1.3 % vs Control) and HPD-PEG (-1.1 %VFA vs HPD, and −0.8 % vs Control). Butyric acid concentrations (when expressed as mM) were highest in HPC-PEG, lowest in Control and HPD-PEG, and showed intermediate values in HPC and HPD; the same was observed when butyric acid was expressed as %VFA although the difference between HPD-PEG with HPC and HPD was not statistically significant. All treatments (HPC, HPD, HPC-PEG, HPD-PEG) had lower concentrations of total VFA than the Control (-2.2, −2.3, −3.1, −8.3 mM, respectively); difference between HPC and HPD were not significant, but HPD-PEG had less total VFA than HPC (-6.1 mM), HPD (-6 mM) and HPC-PEG (-5.2 mM). HPC and HPD had significantly more caproic acid (+0.02 %VFA) when compared with Control, HPC-PEG, and HPD-PEG. The acetic: propionic ratio was highest in HPC-PEG and HPD-PEG (2.11–2.12), lowest in HPC and HPD (1.98–1.99), and showed intermediate values in the Control (2.06).

### *3.2.2. Effect of tannin profile in goat willow (GW) on in vitro gas and $CH_4$ production*

Goat willow had a significant effect on gas and $CH_4$ production on gas yield (mL/g substrate and mL/g digested DM; $P < 0.001$), gas yield ($P < 0.001$), b (h-1) ($P < 0.001$), c (h-1/2) ($P < 0.001$), lag time (h) ($P < 0.001$), A (mL) ($P < 0.001$), gas yield per g substrate degraded (mL/g) ($P < 0.001$), and μ (h-1) ($P < 0.001$) (Table 7).

When compared with Control, treatments HPC, HPD, HPC-PEG, and HPD-PEG had lower gas yield when expressed as mL/g substrate (-44, −51, −20, −31 mL, respectively). Also, HPC and HPD had lower gas yields compared with HPC-PEG and HPD-PEG (-24 and −20 mL, respectively), whilst HPD and HPD-PEG had lower gas yields than HPC and HPC-PEG (-7 and −11 mL respectively).

When compared with Control, treatments HPC, HPD, HPC-PEG, and HPD-PEG had higher gas yield when expressed as mL/g digested DM (+59, +26, +84, +45 mL, respectively). Also, HPC and HPD had lower gas yields compared with HPC-PEG and HPD-PEG (-25 and −19 mL, respectively), whilst HPD and HPD-PEG had lower gas yields than HPC and HPC-PEG (-33 and −39 mL respectively). The results were similar when the variable gas yield per g substrate degraded (mL/g) was estimated based on the model of (France et al., 1993).

When compared with Control, treatments HPC, HPD, HPC-PEG, and HPD-PEG had lower $CH_4$ yield, when expressed as mL/g substrate (-6.63, −6.65, −4.7, −5.1 mL, respectively). Also, HPC and HPD had lower gas yield compared with HPC-PEG and HPD-PEG (-1.95 and −1.55 mL, respectively); whilst there was no difference between HPC and HPD, or HPC-PEG and HPD-PEG.

When compared with Control, treatments HPC, HPD, HPC-PEG, and HPD-PEG had lower CH4 yield when expressed as mL/g digested DM (+7.6 +4.7, +7.9, +5.3 mL, respectively). Also, HPC and HPC-PEG had lower $CH_4$ yield compared with HPD and HPD-

**Table 4**

Means and standard error (SE) for the effect of the interaction between species (goat willow, oak, maple) and month (June-September) on dry matter (DM) content and degradability (DMd), and condensed tannins (CTs) content and profiles.

| Parameters[a] | Goat Willow | | | | Oak | | | | Maple | | | | SE | *P*-value[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | June n = 10 | July n = 10 | Aug n = 10 | Sept n = 10 | June n = 10 | July n = 10 | Aug n = 10 | Sept n = 10 | June n = 10 | July n = 10 | Aug n = 10 | Sept n = 10 | | |
| DM (g/kg fresh) | 437 | 461 | 508 | 456 | 464 | 466 | 502 | 502 | 513 | 519 | 526 | 487 | 14.5 | ***0.076*** |
| **Degradability** | | | | | | | | | | | | | | |
| DMd (g/kg DM) | 513 | 538 | 591 | 595 | 490 | 477 | 499 | 523 | 494 | 519 | 540 | 545 | 13.4 | 0.120 |
| Soluble losses (g/kg DM) | $281^{cB}$ | $282^{bB}$ | $317^{bA}$ | $344^{bA}$ | $326^{bB}$ | $404^{aA}$ | $417^{aA}$ | $427^{aA}$ | $386^{aB}$ | $393^{bB}$ | $402^{aB}$ | $454^{aA}$ | 10.8 | **0.003** |
| **Condensed tannins (CTs)** | | | | | | | | | | | | | | |
| Total CTs (g/kg DM) | 71.8 | 63.5 | 67.9 | 75.4 | 33.2 | 36.8 | 36.7 | 36.0 | 22.3 | 27.3 | 23.9 | 22.8 | 4.79 | ***0.062*** |
| Procyanidins (% CTs) | 54.9 | 54.3 | 55.5 | 54.3 | 46.9 | 44.2 | 45.6 | 44.2 | 97.4 | 98.7 | 98.7 | 99.3 | 7.65 | 0.419 |
| Prodelphinidins (% CTs) | 45.1 | 45.7 | 44.5 | 45.7 | 53.1 | 55.8 | 54.4 | 55.8 | 2.62 | 1.28 | 1.33 | 0.70 | 7.647 | 0.419 |
| *cis* (% CTs) | 30.7 | 27.9 | 26.7 | 28.0 | 38.5 | 37.4 | 30.1 | 37.4 | 93.8 | 96.3 | 94.6 | 95.9 | 4.14 | 0.768 |
| *trans* (% CTs) | $69.3^{a}$ | $72.1^{a}$ | $73.3^{a}$ | $72.0^{a}$ | $61.5^{a}$ | $62.6^{a}$ | $69.9^{a}$ | $62.6^{a}$ | $6.18^{b,A}$ | $3.75^{b, B}$ | $5.43^{b, B}$ | $4.13^{b, B}$ | 4.137 | **0.011** |
| mDP | 5.07 | 4.90 | 4.57 | 4.71 | 4.04 | 3.57 | 3.11 | 3.81 | 5.56 | 4.54 | 4.80 | 5.04 | 0.388 | 0.129 |

[a] DM = Dry matter; DMd = Dry matter degradability; mDP = mean degree of polymerisation.

[b] Significances were declared at $P < 0.05$. Significant differences between dietary treatments within months are indicated with different lower case superscript letters and between months within dietary treatment are indicated with different uppercase superscript letters according to Fisher's Least Significant Difference test ($P < 0.05$).

**Table 5**

Means and standard error (SE) for the effect of the interaction between plant fractions (leaves, twigs) and month (June-September) on dry matter (DM) content and degradability (DMd), and condensed tannins (CTs) content and profiles.

| Parameters[a] | June | | July | | August | | September | | SE | *P*-value[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves n = 15 | Twigs n = 15 | Leaves n = 15 | Twigs n = 15 | Leaves n = 15 | Twigs n = 15 | Leaves n = 15 | Twigs n = 15 | | |
| DM (g/kg fresh) | 425 | 518 | 437 | 528 | 460 | 565 | 423 | 541 | 9.7 | 0.194 |
| **Degradability** | | | | | | | | | | |
| DMd (g/kg DM) | 612 | 386 | 624 | 399 | 655 | 432 | 665 | 444 | 10.2 | 0.305 |
| Soluble losses (g/kg DM) | 377 | 285 | 406 | 314 | 421 | 337 | 444 | 372 | 8.3 | 0.508 |
| **Condensed tannins (CTs)** | | | | | | | | | | |
| Total CT (g/kg DM) | 47.4 | 37.4 | 47.7 | 37.4 | 44.7 | 40.9 | 50.5 | 39.0 | 3.06 | 0.184 |
| Procyanidins (% CTs) | 70.2 | 62.6 | 70.1 | 61.3 | 71.1 | 62.1 | 69.8 | 62.1 | 4.52 | 0.908 |
| Prodelphinidins (% CTs) | 29.8 | 37.4 | 29.9 | 38.7 | 28.9 | 37.9 | 30.2 | 37.9 | 4.52 | 0.908 |
| *cis* (% CTs) | 55.8 | 52.9 | 54.2 | 53.5 | 49.3 | 51.6 | 52.8 | 54.8 | 2.60 | 0.303 |
| *trans* (% CTs) | 44.2 | 47.1 | 45.8 | 46.5 | 50.7 | 48.4 | 47.2 | 45.2 | 2.60 | 0.753 |
| mDP | $5.08^{a}$ | 4.70 | $4.28^{b}$ | 4.38 | $3.91^{c,B}$ | $4.41^{A}$ | $4.33^{b}$ | 4.71 | 0.257 | **0.007** |

[a] DM = Dry matter; DMd = Dry matter degradability; mDP = mean degree of polymerisation.

[b] Significances were declared at $P < 0.05$. Significant differences between dietary treatments within months are indicated with different lower case superscript letters and between months within dietary treatment are indicated with different uppercase superscript letters according to Fisher's Least Significant Difference test ($P < 0.05$).

**Table 6**

Least squares means and standard error (SE) for the effect of tannin profile (high-procyanidin, HPC; high-prodelphinidin, HPD) from goat willow on *in vitro* dry matter degradability (IVDMD, g/kg), the associated kinetic extend of ruminal passage rate (RoP) set at 0.04 h and 0.025 h and the concentrations of the volatile fatty acidsss (VFAs, mM and % VFA); when goat willow was incubated with grass silage at 20 % inclusion rate on total dry matter, over 72 h, with or without the addition of 1.7:1 of polyethylene glycol (PEG):tannins ratio.

| | Dietary treatments[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters[b] | Control | HPC | HPD | HPC-PEG | HDP-PEG | SE | P-value[c] |
| **Degradability and kinetics** | | | | | | | |
| IVDMD (g/100 g) | $75.4^{a}$ | $68.3^{b}$ | $67.7^{b}$ | $69.7^{b}$ | $68.0^{b}$ | 0.63 | **< 0.001** |
| RoP at 0.03 h | $32.0^{b}$ | $32.9^{b}$ | $29.8^{c}$ | $34.8^{a}$ | $29.5^{c}$ | 0.31 | **< 0.001** |
| RoP at 0.025 h | $36.2^{b}$ | $36.6^{b}$ | $33.5^{c}$ | $38.5^{a}$ | $33.2^{c}$ | 0.34 | **< 0.001** |
| **Fermentation parameters** | | | | | | | |
| pH | 6.96 | 7.01 | 6.96 | 6.98 | 7.03 | 0.07 | 0.559 |
| Acetic acid (mM) | $42.2^{a}$ | $39.7^{b}$ | $39.7^{b}$ | $39.8^{b}$ | $37.1^{b}$ | 1.09 | **< 0.001** |
| Propionic acid (mM) | $20.5^{a}$ | $20.0^{b}$ | $20.0^{b}$ | $18.7^{c}$ | $17.6^{c}$ | 0.46 | **< 0.001** |
| Butyric acid (mM) | $4.51^{c}$ | $5.27^{b}$ | $5.19^{b}$ | $5.73^{a}$ | $4.42^{c}$ | 0.347 | **< 0.001** |
| Valeric acid (mM) | 0.96 | 0.99 | 0.99 | 0.94 | 0.93 | 0.047 | 0.835 |
| iso-Butyric acid (mM) | 0.68 | 0.67 | 0.67 | 0.64 | 0.65 | 0.025 | 0.722 |
| iso-Valeric acid (mM) | 1.03 | 1.01 | 1.01 | 0.98 | 0.99 | 0.056 | 0.957 |
| Caproic acid (mM) | 0.13 | 0.14 | 0.14 | 0.13 | 0.12 | 0.010 | 0.313 |
| Total VFA (mM) | $70.0^{a}$ | $67.8^{b}$ | $67.7^{b}$ | $66.9^{b}$ | $61.7^{c}$ | 1.47 | **< 0.001** |
| Acetic acid (% VFA) | $60.3^{a}$ | $58.6^{b}$ | $58.6^{b}$ | $59.4^{b}$ | $60.0^{b}$ | 0.51 | **0.004** |
| Propionic acid (% VFA) | $29.3^{a}$ | $29.5^{a}$ | $29.6^{a}$ | $28.0^{b}$ | $28.5^{b}$ | 0.24 | **< 0.001** |
| Butyric acid (% VFA) | $6.45^{c}$ | $7.77^{b}$ | $7.66^{b}$ | $8.56^{a}$ | $7.16^{b}$ | 0.549 | **< 0.001** |
| Valeric acid (% VFA) | 1.37 | 1.46 | 1.46 | 1.40 | 1.51 | 0.074 | 0.102 |
| iso-Butyric acid (% VFA) | 0.97 | 0.99 | 0.99 | 0.96 | 1.05 | 0.041 | 0.227 |
| iso-Valeric acid (% VFA) | 1.47 | 1.49 | 1.49 | 1.46 | 1.60 | 0.090 | 0.633 |
| Caproic acid (% VFA) | $0.19^{b}$ | $0.21^{a}$ | $0.21^{a}$ | $0.19^{b}$ | $0.19^{b}$ | 0.015 | **0.048** |
| A:P ratio | $2.06^{b}$ | $1.99^{c}$ | $1.98^{c}$ | $2.12^{a}$ | $2.11^{a}$ | 0.020 | **< 0.001** |

[a] HPC = High-procyanidins; HPD, high-prodelphinidins; HPC-PEG = High procyanidins - polyethylene glycol; HDP-PEG = High prodelphinidins - polyethylene glycol.

[b] IVDMD = In vitro dry matter degradability; RoP = Extent of degradation in rumen proper for given rate of passage at 0.04 h and 0.025 h (France et al., 1993); VFA = Volatile fatty acid; A:P = Acetate: Propionate.

[c] Significances were declared at $P < 0.05$. Significant differences between dietary treatments within variable are indicated with different superscript letters according to Tukey's Honestly Significant Difference test ($P < 0.05$).

PEG (-2.9 and −2.6 mL, respectively), whilst HPC and HPD were not statistically different from HPC-PEG and HPD-PEG respectively. The results were similar when the variable gas yield per g substrate degraded (mL/g) was estimated based on the model of (France et al., 1993).

**Table 7**

Least squares means and standard error (SE) for the effect of tannin profile (high-procyanidin, HPC; high-prodelphinidin, HPD) from goat willow on *in vitro* gas production and associated kinetics parameter estimates; when goat willow was incubated with grass silage at 20 % inclusion rate on total dry matter, over 72 h, with or without the addition of 1.7:1 of polyethylene glycol (PEG):tannins ratio.

| | Dietary treatments[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters[b] | Control | HPC | HPD | HPC-PEG | HDP-PEG | SE | P-value[c] |
| **Gas production (GP)** | | | | | | | |
| Gas yield (mL/g substrate) | $164^{a}$ | $120^{d}$ | $113^{e}$ | $144^{b}$ | $133^{c}$ | 0.95 | **< 0.001** |
| Gas yield (mL/g digested DM) | $151^{e}$ | $210^{b}$ | $177^{d}$ | $235^{a}$ | $196^{c}$ | 1.91 | **< 0.001** |
| b (h−1) | $0.06^{c}$ | $0.07^{a}$ | $0.06^{bc}$ | $0.07^{ab}$ | $0.05^{d}$ | 0.002 | **< 0.001** |
| c (h−1/2) | $-0.25^{b}$ | $-0.24^{b}$ | $-0.25^{b}$ | $-0.21^{a}$ | $-0.19^{a}$ | 0.008 | **< 0.001** |
| Lag time (h) | $4.14^{a}$ | $2.92^{c}$ | $3.82^{b}$ | $2.38^{d}$ | $3.06^{c}$ | 0.063 | **< 0.001** |
| A (mL) | $123^{c}$ | $149^{b}$ | $127^{c}$ | $169^{a}$ | $145^{b}$ | 1.343 | **< 0.001** |
| Gas Yield per g substrate degraded (mL/g) | $164^{d}$ | $218^{b}$ | $188^{c}$ | $243^{a}$ | $214^{b}$ | 2.31 | **< 0.001** |
| μ (h−1) | $0.04^{b}$ | $0.04^{a}$ | $0.04^{b}$ | $0.04^{a}$ | $0.04^{c}$ | 0.001 | **< 0.001** |
| **Methane Production (MP)** | | | | | | | |
| Methane yield (mL/g substrate) | $15.7^{a}$ | $9.07^{c}$ | $9.05^{c}$ | $11.0^{b}$ | $10.6^{b}$ | 0.138 | **< 0.001** |
| Methane yield (mL/g digested DM) | $20.9^{a}$ | $13.3^{c}$ | $16.2^{b}$ | $13.0^{c}$ | $15.6^{b}$ | 0.22 | **< 0.001** |
| b (h−1) | $0.06^{c}$ | $0.10^{a}$ | $0.08^{b}$ | $0.10^{a}$ | $0.08^{b}$ | 0.002 | **< 0.001** |
| c (h−1/2) | $-0.24^{a}$ | $-0.42^{c}$ | $-0.35^{b}$ | $-0.44^{c}$ | $-0.36^{b}$ | 0.009 | **< 0.001** |
| Lag time (h) | $6.04^{b}$ | $6.45^{b}$ | $8.49^{a}$ | $6.25^{b}$ | $8.00^{a}$ | 0.255 | **< 0.001** |
| A (mL) | $17.7^{a}$ | $9.32^{c}$ | $11.8^{b}$ | $9.32^{c}$ | $11.2^{b}$ | 0.16 | **< 0.001** |
| Methane yield per g substrate degraded (mL/g) | $23.5^{a}$ | $13.7^{c}$ | $17.4^{b}$ | $13.4^{c}$ | $16.5^{b}$ | 0.24 | **< 0.001** |
| μ (h−1) | $0.04^{c}$ | $0.05^{a}$ | $0.04^{b}$ | $0.06^{a}$ | $0.05^{b}$ | 0.001 | **< 0.001** |

[a] HPC = High-procyanidins; HPD, high-prodelphinidins; HPC-PEG = High procyanidins - polyethylene glycol; HDP-PEG = High prodelphinidins - polyethylene glycol.

[b] Gas/methane yield (mL/g substrate) = $72^{\text{-h}}$ cumulative gas/methane yield per gram of substrate; b = rate constant ($h^{-1}$), independent of time, influencing the fractional rate of degradation (France et al., 1993); c = rate constant ($h^{-1/2}$), decreasing over time, influencing the fractional rate of degradation (France et al., 1993); A = Asymptote of gas/methane production (mL) (France et al., 1993); Gas/methane yield (mL/g substrate degraded) (France et al., 1993); μ = fractional rate of degradation ($h^{-1}$) in the halfway 50 % of the Asymptote (France et al., 1993).

[c] Significances were declared at $P < 0.05$. Significant differences between dietary treatments within variable are indicated with different superscript letters according to Tukey's Honestly Significant Difference test ($P < 0.05$).

## 4. Discussion

### 4.1. *Effect of tree species on tannin profile*

Research on silvopastoral systems has highlighted the benefits and challenges of incorporating tree fodder into livestock diets (Jose, 2009). The tannin profile parameters varied between the tree species investigated in this study and this may directly influence the suitability and effectiveness of these plants as fodder, balancing nutritional benefits against potential drawbacks like reduced palatability or nutrient absorption (Jose, 2009). In the present study, the higher DM, PC, and cis in maple compared with GW and oak, and the lower levels of total CTs, PD, trans, and mDP compared with GW, might contribute to maple's use in traditional medicines and its moderate implications for livestock feeding, potentially due to a balance between nutritional benefits and astringency (Tong et al., 2021). Studies have also shown that environmental conditions and management practices (e.g., soil type, climate, harvesting time) can influence plant phytochemical profiles, including tannin composition (Mueller-Harvey, 2006; Kendall et al., 2021). Key factors like nitrogen availability, carbon:nitrogen ratio, environmental temperature, rainfall, and $CO_2$ concentrations, may affect tannin concentrations in plant species (Lindroth, 2010; Kelln et al., 2021). For example, increased ambient temperature, reduced precipitation, decreased soil fertility, and increased $CO_2$ concentrations have all been reported to increase concentrations of CTs (Tharayil et al., 2011). In addition to the environmental influences, between-species and within-species differences in tannin types and production can occur depending on the stage of maturity and the plant tissue (McSweeney et al., 1999; Schweitzer et al., 2008). These combined factors drive variations in CTs production, influencing the nutritional value of tree fodder across studies.

Previous work has shown that the tannin content and composition can significantly affect herbivore feeding behaviour, digestive efficiency, and health (Hagerman and Butler, 1981; Barry and McNabb, 1999). For instance, higher procyanidins in maple could relate to its use in traditional medicines and potentially beneficial effects on livestock health when included in small quantities in the diet (Barry and McNabb, 1999), including antioxidant properties, essential for reducing animal oxidative stress (Ortuño et al., 2021a). The higher presence of cis configurations may alter tannins' protein binding in the digestive system, impacting their bioavailability, absorption, and metabolism compared to trans configurations (Hagerman and Butler, 1981; Barry and McNabb, 1999).

The elevated levels of total CTs and their mDP in GW suggest that these species could be particularly effective at protein precipitation and may induce more pronounced antimicrobial and anthelmintic (anti-parasitic) effects, beneficial for ruminant health (Jayanegara et al., 2015; Molina-Botero et al., 2019). However, the higher mDP might also reduce the digestibility of these tannins and their associated nutrients (Hagerman and Butler, 1981; Tahvanainen et al., 1985). The prevalence of trans configurations could also influence the structure-activity relationships of these tannins, potentially making them less bioavailable but possibly more stable, as trans configurations in tannins are associated with stronger and more stable binding to proteins due to their linear and less sterically hindered structure compared to the cis configurations (Hagerman and Butler, 1981; Mueller-Harvey et al., 2019a). This characteristic could be related to its ecological role in protecting the plant from being grazed, but also provides a benefit towards sustainable livestock feed systems due to its ability to moderate rumen degradability of protein and potential effect to reduce rumen methanogenesis (Hagerman and Butler, 1981).

Research has shown that certain willow varieties could offer digestible nutrient profiles suitable for ruminant nutrition, although the study emphasises the need for specific evaluations (Moreira et al., 2013). The role of tannins in plant-animal interactions has shed light on how these compounds can influence feed digestibility (Mueller-Harvey, 2006; Jayanegara et al., 2015; Mueller-Harvey et al., 2019a). While high concentrations of tannins typically reduce DMd, the specific tannin structure, type, and interaction with other dietary components can modulate this effect (Besharati et al., 2022). For GW, tannins with less adverse impact on digestibility or even protective effects on dietary proteins could explain its higher DMd (Hagerman and Butler, 1981). Differences in these results may have been due to the chemical composition between GW and the other two species, including tannin concentrations and chemical structure. It is generally accepted that a dietary CTs content at a low to moderate level (2–4 % of dry matter) in animal feed is beneficial for animal nutrition on protein metabolism in ruminants, reduces bloat, and has an anthelmintic effect on gastrointestinal parasites, although this threshold value may vary depending on the tannin source and structure, diet composition and animal species (Patra and Saxena, 2011; Besharati et al., 2022). In practice, these differences in DMd, chemical composition, and tannin content may be related to palatability and feed intake (Tong et al., 2021). For example, feed intake reduction has been observed with increasing dietary CTs in previous studies using diet from lespedeza reduced feed intake and $CH_4$ emissions in goats (Animut et al., 2008) probably due to reduced palatability caused by the astringent nature of tannins and the slowing of ruminal digestion, thereby increasing the filling effect (Kaitho et al., 1997; Frutos et al., 2004). Feed intake can be influenced by the concentrations of CTs in the diet with $> 50$ g $\mathrm{CTs \cdot kg^{-1}}$ DM consistently decreasing feed intake (Mueller-Harvey et al., 2019a). This may also be livestock species-specific because diet selection, microbial species, and physiological differences between species may play a role in the passage rate of feed particles through the rumen and, thus, the time available for CTs to impact digestibility (Waghorn et al., 1987; Tharayil et al., 2011).

Oak has been documented to have higher PD content, which indicates a strong potential for astringency and bitter taste, which could impact palatability for livestock (DemİR and Kamalak, 2024). Prodelphinidins are known for their potent antioxidant activity and role in plant defence mechanisms. For livestock, these compounds might offer health benefits, including reduced gastrointestinal parasites and improved protein utilisation, but at the risk of reduced feed intake due to astringency (Mueller-Harvey et al., 2019a; Ortuño et al., 2021b). Oaks' tannin profile significantly impacts ruminant nutrition, potentially reducing palatability but offering benefits regarding parasite control and $CH_4$ emission reduction (DemİR and Kamalak, 2024).

Tannin concentrations, mDP, and the ratio of *cis*:*trans* and PC:PD flavan-3-ol units determine the biological activity of CTs in animals, including antimicrobial activity, anti-parasitic, and antioxidant properties (Mueller-Harvey et al., 2019a; Tong et al., 2021). Therefore, comprehensive tannin profiling is necessary to understand the mechanism of action of these compounds to support the appropriate management of tree foliage for animal nutrition. Additionally, periodically examining CTs concentrations while evaluating animal performance and physiological status can help improve understanding of the influence of CTs concentrations in willow, oak, and maple foliage on animal production and diet composition (Ortuño et al., 2021b).

### 4.2. *Effect of plant fractions on tannin profile*

The effect of tannins on plant fractions, particularly leaves and twigs, across different species has been researched with a focus on ecological interactions, plant defence mechanisms, decomposition rates, and impacts on nutrient cycling (Mora Izquierdo et al., 2011). In this study, the presence of higher levels of CTs in leaves compared to twigs is consistent with previous work, which suggests that parts of the plant that are more valuable and vulnerable to grazing (such as leaves, which are crucial for photosynthesis) are often more heavily defended (Barbehenn and Peter Constabel, 2011). Tannins serve as a chemical defence mechanism against herbivores by reducing palatability and interfering with the digestion of plant material (Barbehenn and Peter Constabel, 2011). McAllan (1992) suggests that plant fractions (leaves, stems, flowers, seeds) are the main drivers for tannin concentrations, alongside other genetic (species, genotype) and environmental factors (growth stage, climate, soil type, light exposure, water stress, nutrient availability); and that plant fractions can vary significantly in their tannin content. Stressful conditions often lead to increased tannin concentrations as part of the plant's defensive response (Barbehenn and Peter Constabel, 2011). Makkar (2003) has reported the inverse relationship between tannin content and degradability due to tannins binding to proteins and other macromolecules, forming complexes that are less easily digested by herbivores. This can explain the lower final DM degradability observed in leaves with higher tannin concentrations. The mDP of tannins influences their binding affinity and the strength of the interactions with proteins (Schmidt et al., 2012). In this study, lower mDP in leaves suggests that these tannins may be less polymerised, potentially weakening their efficiency in forming protein-tannin complexes, which could allow better access and efficacy of the digestive enzymes in the gut and increase the digestibility and nutritional quality of the leaves (Hagerman and Butler, 1981).

The differential allocation of tannins and other biochemical compounds between leaves and twigs is an adaptation that balances the plant's need for growth, reproduction, and defence (Kumar and Horigome, 1986). While the higher concentrations of tannins in leaves are a protective measure against herbivory, they also impact the nutritional quality of these plant fractions for animals that feed on them (McAllan, 1992). At the farm level, understanding these patterns is crucial for selecting fodder that optimises nutritional value, and it is recommended that DMd, chemical composition, and CTs content and profiling should be accounted for in ration formulation.

### 4.3. Seasonal variation on tannin profile

Plant tannin content profiles vary seasonally between organs and tissues and in response to many biotic and abiotic factors, including nutrient content, water availability, $CO_2$ levels, light availability, and temperature. The increase in DMd and soluble losses towards the end of the growing season could be attributed to plants entering a senescence phase, where cell wall breakdown and nutrient mobilisation occur, enhancing digestibility (Bryant et al., 1983; Herms and Mattson, 1992; Kingston-Smith et al., 2013). The increase in DM from June to August reflects active growth phases fuelled by optimal photosynthetic conditions (Bryant et al., 1983). Understanding the timing of peak digestibility for the different forages and fodders is crucial for optimising livestock feeding strategies and ensuring high-quality feed is provided. For livestock management, this suggests that forage harvested in early autumn might offer higher nutritional value but also indicates a potential increase in the rate of decomposition of plant litter, impacting nutrient cycling in ecosystems.

As the season progresses, resources may shift towards growth and seed production, possibly reducing tannin complexity (Salminen et al., 2011). Understanding the defensive role of tannins and their seasonal variations is essential for managing grazing to minimize the impact of anti-nutritional factors on livestock (Bryant et al., 1983). Early-season higher mDP in tannins might reflect a defensive strategy against herbivores during the time of the year when plants are more vulnerable (Barbehenn and Peter Constabel, 2011). The higher *cis* and lower *trans* configurations of flavanols in maple compared to GW reflect underlying differences in their secondary metabolite synthesis pathways. Tannin configurations significantly influence their chemical properties, including solubility, stability, and reactivity with proteins, as well as their antioxidant capacity and biological activity. Trans flavanols are more stable and less reactive than cis flavanols due to their spatial bond arrangements, impacting their biological roles in plants (Rana et al., 2006; He et al., 2008; Zhang et al., 2020). Previous studies have highlighted that the synthesis of flavanol configurations can be influenced by various factors, including genetics, environmental stress, and developmental stage (Cheynier et al., 2013). For example, studies on different tree species have shown that environmental stresses such as UV exposure and water scarcity can increase flavanol production, altering *cis*-to-*trans* ratios based on specific stressors (Cheynier et al., 2013). Early-season tannins in tree fodder are more complex, reflecting defensive strategies against herbivores (Barbehenn and Constabel, 2011). Species-specific pathways, as seen in maple and GW, further demonstrate how genetic and environmental factors shape flavanol synthesis, influencing chemical properties and ecological roles (Cheynier et al., 2013). Aligning grazing practices with periods of lower tannin concentrations can enhance livestock health and sustainable agriculture.

### 4.4. Effect of tannin profile in goat willow on in vitro dry matter degradability (IVDMD) and rumen fermentation parameter

In this study, the IVDMD of GW was significantly reduced, likely due to its specific composition and potentially lower lignin content compared to other trees. In this study, CON also showed higher acetic acid, propionic acid, and total VFA in comparison to HPD-PEG, indicating a higher degradability than tree fodder (Min et al., 2003). This is in contrast to the results Jayanegara et al. (2015) reported that certain tree fodder, such as mimosa and quebracho, have a beneficial effect on digestibility due to their unique structural and chemical properties. Tannins typically reduce IVDMD by binding with dietary proteins and enzymes, inhibiting their digestion in the gut. However, when tannins were neutralised by PEG and tree fodder was used, there was still a reduced IVDMD, which indicates that there are factors beyond the impact of tannins that can reduce IVDMD in diets containing GW. This also aligns with previous findings (Min et al., 2003) that showed the addition of PEG typically leads to an increase in IVDMD by neutralising the negative effects of tannins. This indicates that tannins are a significant factor in reducing digestibility when they are present in high concentrations in forages.

This study showed that different types of tannins (PC vs PD) can affect fermentation patterns differently (Patra and Saxena, 2011). HPC's association with higher RoP and butyric acid production suggests it may favour a different microbial population or fermentation pathway than HPD. A previous study showed the acetic:propionic (A:P) ratio is an important indicator of ruminal fermentation efficiency and $CH_4$ production (Beauchemin et al., 2020). Lower A:P ratios, observed in GW diets during incubation generally indicate more efficient energy use and less $CH_4$ output. The observed variations in VFA profiles suggest that tannin type can significantly reduce A:P ratio, whilst the significant effect of PEG addition also indicates that the observed differences are due to the tannin content.

The reduction in total VFA production and changes in the proportions of acetic, propionic, and butyric acids suggested that GW may alter rumen fermentation patterns. This finding is consistent with the previous studies indicating that diets enriched with *Acacia spp*., *Mimosa spp*., *Quercus spp*., and *Calliandra calothyrsus* can modify VFA production and affect energy metabolism in ruminants (Patra and Saxena, 2011). A lower A:P ratio generally indicates more efficient energy use and less $CH_4$ production, a desirable outcome for reducing GHG emissions from livestock (Beauchemin et al., 2020). Previous studies also mentioned tree fodder's potential to improve feed efficiency and alter VFA profiles can help in formulating more balanced diets that enhance animal performance and health (Mueller-Harvey et al., 2019b; Beauchemin et al., 2020). The use of GW and similar tree fodder could be strategically increased in grazing systems to utilise their beneficial effects on rumen fermentation, such as improving nitrogen utilisation, reducing $CH_4$ emissions, and enhancing overall animal health. This approach could potentially reduce reliance on synthetic feed additives and support more natural feeding regimes (Kelln et al., 2021).

### 4.5. Effect of tannin profile in goat willow on in vitro gas and $CH_4$ production

Similar to the present work, previous studies have shown that tannins, including PC and PD, can potentially reduce $CH_4$ production by directly inhibiting the microbial population responsible for $CH_4$ synthesis, for example, suppressing the activity and proliferation of methanogenic archaea and other fermentation-related microorganisms (Tavendale et al., 2005). Beauchemin and McGinn (2006) reported how various additives, such as fats, oils, and tannins, reduce $CH_4$ and total gas production in the rumen, providing a baseline understanding of how different nutritional approaches like the inclusion of GW in ruminant diets might perform. Their findings align with the current study, suggesting that feed additives, including GW, can effectively modify rumen fermentation and reduce $CH_4$ emissions.

Grass silage (CON), typically low in tannins, does not have the same inhibitory effects on rumen microorganisms as tannin-rich diets. Therefore, it often results in higher overall fermentation activity, leading to greater gas and $CH_4$ production (Johnson and Johnson, 1995). The role of PEG in binding with tannins to mitigate their anti-nutritional effects is well-documented; by complexing with PD, PEG can neutralise the more potent tannin effects, potentially altering fermentation patterns compared to diets without PEG (Lamy et al., 2011). Notably, when PC and PD were neutralised by adding PEG in the diets, gas, and $CH_4$ yields were still reduced compared with the CON diet in the current study, but these measurements were higher than HPC and HPD diets without PEG. This indicated that the inhibitory effect of GW on gas and $CH_4$ production may be partly, but not entirely, due to tannins. Based on the findings that gas and $CH_4$ yields were higher in tannin-containing diets when expressed per g digested DM, the lower overall degradability of the HPC and HPD diet appears to also be a critical factor.

The rate constants 'b' and 'c', lag time, and 'μ' (rate of microbial growth) are critical for understanding how quickly and efficiently feed substrates are fermented in the rumen. Research has shown that feeds influencing these parameters can alter the speed and efficiency of nutrient breakdown (Getachew et al., 1998). Adjusting diets to optimise these kinetic parameters can enhance animal growth rates and feed efficiency, leading to economic benefits in livestock production. This involves improving the diet to achieve the most efficient and advantageous results regarding animal growth and feed utilisation. Lower values, which are observed for the GW diets and even lower in HPC than HPD, indicate a less efficient conversion of feed into fermentable gases, which is undesirable for energy extraction but may have trade-offs with environmental benefits such as the lower rates of $CH_4$ synthesis and protein degradation in the rumen (Patra and Saxena, 2011). Similar to the present study, previous work has shown that CON leads to faster initiation of fermentation due to the rapid availability of fermentable carbohydrates compared to diets containing tree fodder such as GW, which have higher fibre content and lower fermentable carbohydrate availability. Tannins in diets like those with HPC and HPD are known to initially slow the fermentation process by binding to proteins and possibly inhibiting microbial enzymes (McAllister et al., 1994). Adding PEG, which creates a complex with PC and PD, to tannin-rich diets can negate the effects of tannins by binding to them, reducing their ability to bind with proteins, restoring the microbial activity and fermentation rates closer to those observed in tannin-free diets, and mitigating potential anti-nutritional effects (Makkar et al., 1993; Patra and Saxena, 2011). In the present study, CON had higher GP lag time, MP gas yield, MP c, MP A, and MP gas yield per g substrate degraded, even compared with GW diets containing PEG; thus revealing that there may be other nutritional parameters in these diets that may have reduced the speed of fermentation, beyond the direct impact of tannins. The observed increase in lag times between GW diets containing or not PEG suggests that PEG may not completely negate the effects of HPC and HPD on the fermentation characteristics (Makkar et al., 1993).

Understanding these dynamics that tree fodder incurs in livestock diets can help formulate rations and adapt grazing management that optimises fermentation efficiency while minimising $CH_4$ emissions, striking a balance between environmental sustainability and animal performance. While PEG is used to counteract tannin effects, its efficacy can vary based on the type of tannin, suggesting a need for tailored strategies when using PEG in a tannin-rich diet (Min et al., 2003). The present study showed that the effect of PC and PD is highly variant, with HPC producing more gas and less $CH_4$ than HPD, although the latter is true only when $CH_4$ was presented per g digested DM. PC generally have a less pronounced effect on inhibiting microbial activity compared to PD, which are known for their stronger binding affinities and higher anti-microbial properties (Patra and Saxena, 2009). This is because PC, containing fewer hydroxyl (-OH) groups, form weaker hydrogen bonds with proteins and microbial enzymes, leading to less interference with microbial activity and fermentation processes. On the other hand, PD have more hydroxyl groups, resulting in stronger and more extensive hydrogen bonding with proteins and enzymes. This stronger binding affinity of PD inhibits microbial growth and enzyme function more effectively, reducing the overall microbial activity and fermentation efficiency. Consequently, while PC allow for more effective fermentation and higher gas production, PD's higher anti-microbial properties lead to a more significant reduction in $CH_4$ synthesis, especially when measured per unit of digested DM (Min et al., 2003; Patra and Saxena, 2009).

A key limitation of this study is the use of rumen fluid from a single donor cow, due to constraints of access to donor animals at the time of the experiment, which may affect reproducibility by not accounting for inter-animal variation. Ideally, rumen fluid from at least two animals should be used (Yáñez-Ruiz et al., 2016). However, ensuring that all treatments were exposed to the rumen fluid from the same donor collected at the same time, allowed for valid comparative trends. While this limitation affects reproducibility, the findings still offer valuable insights into dietary effects on rumen fermentation and provide a foundation for future studies incorporating multiple donor animals to enhance reliability.

## 5. Conclusions

The present study demonstrates that dry matter degradability (DMd) and tannin profile of tree fodder varies with tree species, plant fractions, and season. There was a 2.9-fold variation in total CTs, a 2.2-fold variation in procyanidins (PC) content, and a 37.0-fold variation in prodelphinidins (PD) content between goat willow (GW), oak, and maple. Considering the diverse effects of different tannin types on animal metabolism, e.g., PC is known to reduce $CH_4$ emissions whilst PD is known to reduce the burden of gastrointestinal parasites, it is important to consider tannin variation (both content and profile) when incorporating tree fodder into animal diets. This can maximise the positive impacts, depending on the target of the dietary intervention, while minimising the potential adverse nutritional effects (e.g., by avoiding over-feeding CTs beyond 20 % of diet dry matter). In some species (e.g., oak and maple), the PC and PD contents vary between leaves and twigs, notably in a contrasting manner, which may also need to be accounted for when tree fodder is used to deliver CTs in animal diets. As $CH_4$ mitigators, both High-PC and High-PD tree fodder reduced $CH_4$ yield by up to 42 % per g substrate, or up to 36 % per g degraded DM, in an *in vitro* gas production system, with High-PC showing a stronger effect. This effect was partly due to CTs (e.g., $CH_4$ reduction was observed even when tannins were neutralised with polyethylene glycol) but also due to the reduced degradability of tree fodder than the grass silage that was replaced in the diet. The findings highlight the potential tannin-containing tree fodder can play in feeding regimes for sustainable livestock feeding practices, whilst comprehensive tannin profiling and subsequent dietary adjustments are essential to maximising the potential beneficial impact. These benefits may contribute towards resilient silvopastoral systems to reduce climate impact and improve animal health and production.

## CRediT authorship contribution statement

**Crompton Les A.:** Writing – review & editing, Validation, Software, Methodology, Formal analysis, Data curation. **Theodoridou Katerina:** Writing – review & editing, Methodology. **Stergiadis Sokratis:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Sari Nurul Fitri:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Ray Partha:** Writing – review & editing, Supervision. **Rymer Caroline:** Writing – review & editing, Supervision. **Smith Jo:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Natalello Antonio:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Kliem Kirsty E.:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Whistance Lindsay:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Christodoulou Christos:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation.

## Declaration of Competing Interest

The authors declare no conflict of interest

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