Characterisation of the nutritional composition of plant components of a herb-clover mix during November to May in New Zealand

WEMLJ Ekanayake*, RA Corner-Thomas, LM Cranston, RE Hickson, PR Kenyon and ST Morris

School of Agriculture and Environment Science, Private Bag 11-222, Massey University, Palmerston North, 4442, New Zealand

**Corresponding author*. Email: jayampathiekn@gmail.com

Abstract

Animal performance on herb-clover mixes (containing plantain, chicory, red clover and white clover) can be inconsistent. Potentially this could be due to variation in botanical and nutritional composition of plant components affecting animal selectivity and, thus, performance. Herbage samples were collected monthly from November 2016 to May 2017, from 3 paddocks located at Massey University's Keeble farm, Palmerston North, New Zealand. Quadrat-cut samples were used to determine the herbage mass and botanical composition. Hand-plucked samples of each plant components were analysed for the nutritional composition. The botanical composition of the herb-clover mix differed over time ($P<0.05$). The percentage of chicory stems increased (9.2 to 16.2%) and plantain stems decreased (14.7 to 1.0%, $P<0.001$). Overall, the crude protein and metabolisable energy of white clover, red clover, plantain and chicory leaves were higher ($P<0.05$) than those of plantain and chicory stems in each month except in November. These data indicate that a higher proportion of chicory and plantain stems in a herb-clover mix would reduce overall metabolisable energy and crude protein. This information can be useful for farmers to manipulate both the botanical and nutritional composition of herb-clover mix to optimise animal performance.

Keywords: herb-clover mix; nutritional composition; high-energy plant components

Introduction

Recently, in New Zealand, the use of herb-clover mixes containing plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) has increased. This has been driven by improved sheep performance (ewe and lamb liveweight gains pre-weaning, ewe milk production, lamb growth after weaning) on herb-clover mix compared to that observed on perennial ryegrass (*Lolium perenne* L.) and clover based pasture during spring to autumn period (Cranston et al. 2015; Ekanayake et al. 2017). Liveweight gains of lambs and ewes after weaning on herb-clover mixes, however, have been reported to be inconsistent across years (Corner-Thomas et al. 2018; Ekanayake et al. 2018). These inconsistencies may be due to the variation in botanical composition, nutritional profile, palatability and animal selectivity of plant components in the herb-clover mix.

The nutritional profile of chicory and plantain, grown as monocultures, has been reported previously (Lee et al. 2015; Martin et al. 2017) and shown to vary with nitrogen fertilizer application and rate of defoliation (Martin et al. 2017). Red and white clover improve the nitrogen concentration in the soil, thus, provide bioavailable nitrogen to companion plant species in pasture mixes (Ledgard 2001). The nutritional profile of chicory and plantain, therefore, may differ when grown with red and white clover in a mix sward compared to monoclultures. The nutritional composition of the herb-clover mix has previously only been analysed as a mixed sample (Cranston et al. 2015; Ekanayake et al. 2017). The nutritional composition of the individual plant components within a herb-clover mix and their respective effects on overall nutritive value during November to May period is not known. Understanding the nutritional changes of each plant component of the herb-clover mix may help to explain at least some of the variations in animal performance observed in previous studies.

We hypothesized, therefore, that the nutritional and botanical composition of individual plant components of the herb-clover mix may change during November to May period. Thus, the the nutritional composition and relative nutritional contribution of each plant component in the herb-clover mix was characterised during November to May period.

Materials and methods

Experimental site

The experimental site was at Massey University's Keeble farm, 5 km southeast of Palmerston North (40°24' S and 175°36' E), New Zealand. The soil type at the experimental site was a recent alluvial soil. Five paddocks (a total area of 8.5 ha) of a herb-clover mix containing plantain (cultivar 'Ceres Tonic'), chicory (cultivar 'Puna 2'), red clover (cultivar 'Sensation') and white clover (cultivar 'Bounty') were used. These paddocks were sown during autumn in 2012 and 2013 with a seed mixture of chicory (6 kg/ha), plantain (6 kg/ha), white clover (4 kg/ha) and red clover (6 kg/ha). All paddocks were fertilised in April 2016 and May 2017 with 30% Potash Super (phosphorous 6.3%, potassium 15%, sulphur 7.7% and calcium 14%) basal fertiliser mixture (Ravensdown, New Zealand) at a rate of 400 kg/ha and with urea (Ravensdown, New Zealand) at a rate of 67 kg/ha (30 kg N/ha). Paddocks were treated with Preside herbicide (active ingredient; paraffinic oil 582 g/l and alkoxylated alcohol non-ionic surfactants 240 g/l, Corteva agriscience, New Zealand) in May 2016 and June 2017 to control the spread of broadleaf weeds and with Gallant Ultra (active ingredient; haloxyfop-P at 520 g/litre present as the haloxyfop-P-methyl in the form of an emulsifiable concentrate, Dow AgroSciences, New Zealand) to control the spread of grass. All paddocks were grazed by sheep in a rotational grazing system at 3-4 weekly intervals to a post-grazing residual of ~ 7 cm. Herbage was then allowed to re-grow for 2 weeks and samples were collected. This procedure was followed in each month, therefore, the relative maturity of herbage at the time of sampling would have been consistant and had no effect on the differences of nutritional composition of plant species.

Weather data were received from National Institute of Water and Atmospheric research (NIWA) weather station situated between 40°38' S and 175°61' E. Daily data included maximum and minimum air temperature and rainfall. Those data, however, was not reported in this paper.

Herbage measurements

Herbage samples were collected monthly (11 November 2016, 13 December 2016, 13 January 2017, 16 February 2017, 16 March 2017, 17 April 2017 and 25 May 2017). At each sampling time, herbage samples were collected from three paddocks (of the five paddocks) that were ready to be grazed. Four random quadrat cuts (1.0 m^2 each) were taken to ground level in each of three paddocks using an electric shearing hand-piece (Frame 1993). Samples (1.0 m^2 each) were collected from different locations in a paddock at each sampling, therefore, it was unlikely that sample collection impacted the composition of herbage at next sampling. Herbage samples were then sorted into plantain leaf, plantain stem, chicory leaf, chicory stem, red clover, white clover, weeds and dead matter to estimate botanical composition. They were then oven dried at 60°C in a draught oven to a constant weight to estimate the herbage dry matter. For each paddock, the herbage mass was calculated as the average of four quadrat cuts.

At each sampling time, one hand-pluked sample of each of plantain leaf, plantain stem, chicory leaf, chicory stem, red clover and white clover were randomly collected from the same three paddocks for nutritional-quality analysis. Samples were collected between 9.00 AM and 12.00 PM at each sampling time. Whole leaves of chicory and plantain were collected from a minimum of 10 different plants to create one subsample from each paddock. Red and white clover leaves were collected with stems, but were not separated into leaves and stems. Weed and dead matter were not analysed for nutritional composition.

Nutritional composition

Samples were freeze dried, ground and then passed through a 1 mm sieve. Samples were analysed using *in-vitro* methods to determine the nutritional quality including *in-vitro* organic matter digestibility (OMD), dry matter digestibility (DMD) and digestible organic matter digestibility (DOMD) (Roughan and Holland 1977). Percentage crude protein (CP) was determined by "Dumas" procedure (AOAC method 968.06) using a Leco total combustion method (LECO Corporation, St. Joseph, MI, USA). Percentage neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed by a Tecator Fibretec System (Robertson and Van Soest 1981). Metabolisable energy content (ME) of herbages was calculated using the organic matter digestibility (DOMD×0.16 MJ/kg DM) (Roughan and Holland 1977).

Predicted metabolisable energy

Predicted ME of herb-clover mix (PME) was calculated in order to estimate the relative contribution of individual species to the total metabolisable energy concentration of the herb-clover mix, using the following equation:

PME (MJ/kg DM)= Sum of (ME of the plant component (MJ/kg DM) × propotion of the plant component in the herb-clover mix without weed and dead matter)

Sheep selectively graze high-energy plant species such as chicory, plantain and clover compared to other plant species in herb-clover mix (Cave et al. 2015; Pain et al. 2010). Therefore, in such a mix sheep predominantly fulfilled their nutritional requirements from chicory, plantain and clover but not from weed and dead matter. It was, therefore, assumed that weed and dead matter would only make a minor contribution to the overall consumed ME of the herb-clover mix that a sheep would choose, therefore, they were not included in the PME calculation. Predicted ME of herb-clover mix was calculated using ME of individual plant components and adjusted botanical composition, the composition of herb-clover mix adjusted to 100% without dead matter and weed. Further, Predicted ME of herb-clover mix was calculated both with the ME of plantain and chicory stems included ($PME_{with\text{-}stems}$) and without ($PME_{no\text{-}stems}$). $PME_{no\text{-}stems}$ was calculated as it is possible that animals graze herb-clover mix without choosing chicory and plantain stems.

Statistical analysis

All the data were tested for normality and outliers before analysis. Herbage mass, botanical composition and nutritional composition data were subjected to analysis of variance using the MIXED procedure in SAS (Statistical Analysis System, version 9.2; SAS Institute Inc., Cary, NC, US), and analysed using a mixed model that allowed for repeated measures. Herbage masses were analysed using a model that included sampling month as a fixed effect and paddock as a random effect. The botanical composition was analysed separately with a generalised model using a poisson distribution and logit transformation. Botanical composition and herbage-quality data were analysed using the MIXED procedure, with a model including the fixed effects of sampling month and plant component and the random effect of paddock number.

Results

Comparisions within each nutritional component were undertaken both within (lowercase superscripts; l-p) and across (lowercase superscripts; a-f) months [November (late spring); December, January, February (summer); March, April, May (autumn)] resulting in a number of potential comparisions. Due to the large number of significant differences observed, the following sections outline only the main trends as individual comparitions can be seen in the table.

Botanical composition (%) and herbage mass (kg DM/ha)

The percentage of chicory leaves in the herb-clover mix increased significantly from 30% in November to 50% in March and then decreased to 33% in May (Figure 1). The percentage of chicory stems was higher ($P<0.05$) in December (9%), February (15%), April (11%) and May (8%) than in all other months (5-6.5%). The percentage of plantain leaves increased from 8% in November to 18% in May while plantain stems decreased from 13% in November to 0% in May. Herbage mass of the herb-clover

Figure 1 The botanical composition (% of herbage species) of herb-clover mix during November 2016 to May 2017.

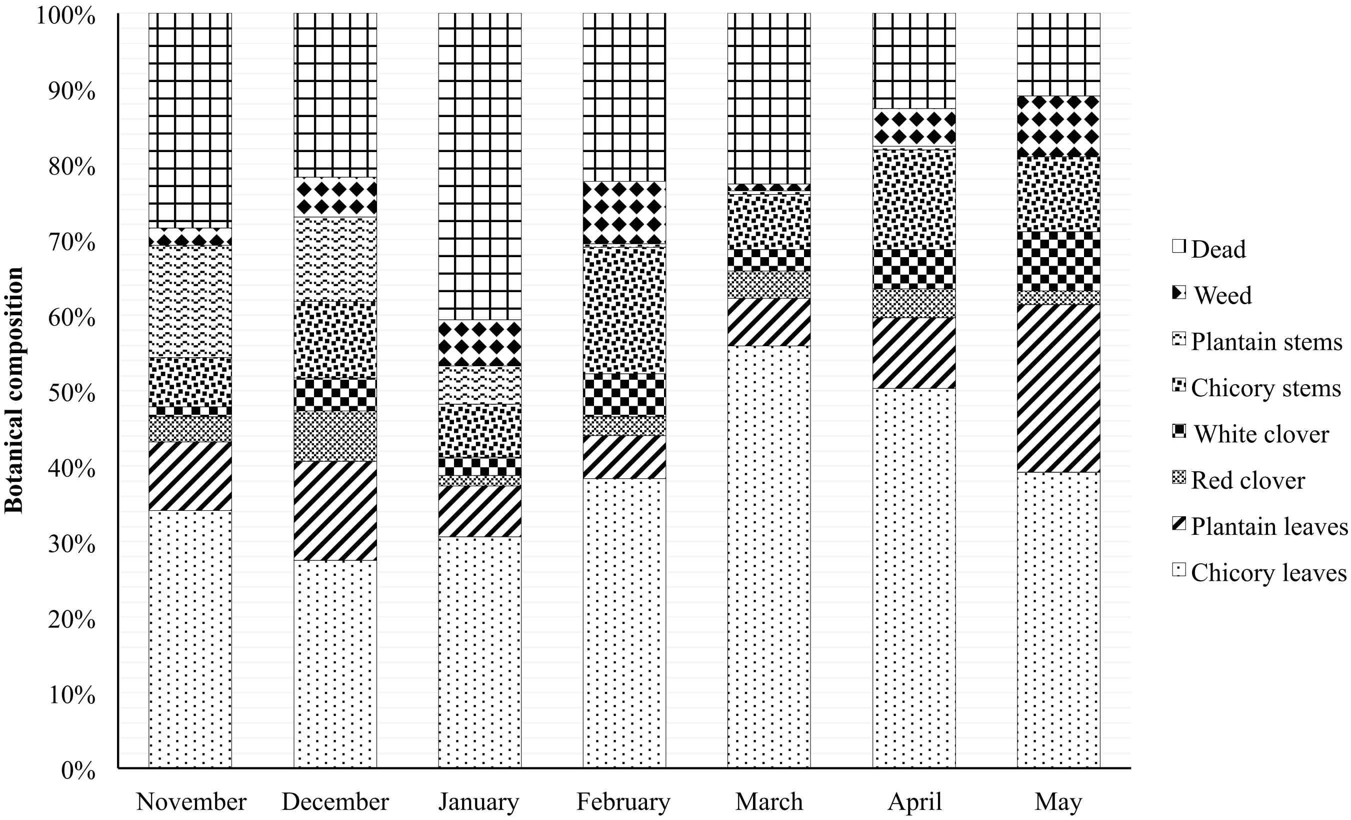

Table 1 Crude protein (CP), and metabolisable energy (ME) in dry matter of plant components in the herb-clover mix during November 2016 to May 2017 (Mean ± SEM).

Plant species/ component	November	December	January	February	March	April	May	SEM
				CP (%)				
Chicory leaf	15.7^{amn}	18.5^{an}	17.8^{amn}	15.4^{an}	19.7^{abno}	17.2^{an}	23.2^{bm}	1.8
Chicory stem	8.8^{bl}	4.2^{abl}	2.8^{al}	2.3^{al}	3.4^{al}	2.1^{al}	1.7^{al}	1.8
Plantain leaf	20.6^{abn}	16.9^{an}	16.7^{am}	16.3^{an}	18.1^{an}	22.3^{bno}	23.0^{bm}	1.8
Plantain stem	12.6^{clm}	7.2^{abm}	6.0^{al}	10.0^{abcm}	11.0^{bcm}	8.4^{abcm}	-	1.8
Red clover	27.5^{bo}	25.3^{bp}	20.4^{amn}	21.8^{ao}	23.3^{abo}	24.9^{abo}	27.7^{bm}	1.8
White clover	28.4^{bo}	22.7^{ao}	22.6^{an}	28.0^{bp}	28.4^{bp}	26.9^{abo}	30.4^{bn}	1.8
				ME (MJ/kg DM)				
Chicory leaf	11.1^{bn}	11.2^{bm}	11.4^{bn}	11.3^{bo}	11.5^{bo}	11.0^{bm}	10.5^{am}	0.1
Chicory stem	10.6^{fm}	9.4^{el}	8.8^{cdl}	8.6^{cdl}	8.5^{cl}	8.1^{bl}	7.2^{al}	0.1
Plantain leaf	11.0^{bmn}	11.0^{bm}	11.1^{bmn}	10.9^{bn}	11.1^{bn}	11.2^{bm}	10.5^{am}	0.1
Plantain stem	9.9^{cl}	9.3^{bl}	9.1^{abl}	9.3^{abm}	10.0^{cm}	8.9^{al}	-	0.1
Red clover	11.2^{bn}	11.4^{bm}	10.9^{bm}	10.8^{bn}	11.3^{bno}	11.1^{bm}	10.3^{am}	0.1
White clover	11.4^{an}	11.1^{am}	11.1^{amn}	11.1^{ano}	11.5^{ao}	11.3^{am}	10.9^{am}	0.1

SEM, standard error of the mean

$^{a-f}$ Means with different superscripts within rows are significantly different across months ($P<0.05$).

$^{l-p}$ Means with different superscripts within columns are significantly different across plant components ($P<0.05$).

mix ranged between 1154±113 to 4821±400 kg DM/ha during November to May period..

Crude protein (%) and ME (MJ/kg DM)

The CP of plant components differed within each month (Table 1). Crude protein of chicory and plantain leaves and red and white clover was higher ($P<0.05$) than that of chicory and plantain stems in each month except in November. When compared the CP of each plant components across months, CP of chicory leaves did not differ ($P>0.05$) during November to April (15-19%) but increased to 23% in May. Crude protein of chicory stem decreased from 9% in November to 1.7% in May. Crude protein of plantain leaves did not differ ($P>0.05$) from November to March (16-20%) but increased to 22% in April and 23% May. Crude protein of plantain stems and white clover was higher ($P<0.05$) in November than in December and January and then increased to an intermediate level in late summer and autumn. Crude protein of red clover was higher ($P<0.05$) in November and December than in January and February and then was intermediate during March to April.

Metabolisable energy of chicory and plantain leaves, and red and white clover was higher ($P<0.05$) than that of chicory and plantain stems in each month except in November. When compared the ME of each plant components across months, ME of chicory leaves did not differ ($P>0.05$) during November to April (11-11.5 MJ/kg DM) but decreased to 10.5 MJ/kg DM ($P<0.05$) in May. Similar trends were observed for plantain leaves and red clover. Metabolisable energy of chicory stem gradually decreased from 10.6 MJ/kg DM in November to 7.2 MJ/kg DM in May. Metabolisable energy of plantain stems in November (9.9 MJ/kg DM) and March (10 MJ/kg DM) was higher ($P<0.05$) than during rest of the time period (8.9-9.3 MJ/kg DM). Metabolisable energy of white clover did not differ ($P>0.05$) over the course of time.

Acid detergent fibre and NDF (%)

Acid detergent fibre of chicory and plantain stems was higher ($P<0.05$) than that of the other plant components in each month except in November (Table 2). When compared across months, the ADF of each plant component of the herb-clover mix, the ADF of chicory leaves was lower ($P<0.05$) in March (13.1%) than in May (19.9%) and November (18.3%) and it was intermediate in all other months. Acid detergent fibre of chicory stems gradually increased from 23.6% in November to 66.3% in May. Acid detergent fibre of plantain leaves was lower ($P<0.05$) in January, March and April than in May and then it was intermediate in all other months. Plantain stems had higher ($P<0.05$) ADF in December, January, February and April than in November and March. Red clover had higher ($P<0.05$) ADF in February and May than in December and it was intermediate during rest of the time period. Acid detergent fibre of white clover was lower ($P<0.05$) in March than in May and it did not differ ($P>0.05$) during the rest of the time period. Neutral detergent fibre of plant components differed as similar as to the differences of ADF of plant components.

Predicted ME (MJ/kg DM)

$PME_{no\text{-}stems}$ was higher ($p<0.05$) during November to March than during April to May (Figure 2). $PME_{no\text{-}stems}$ was higher than $PME_{with\text{-}stems}$ at each sampling month.

Figure 2 Adjusted botanical composition (% of herbage species without chicory and plantain stems) of herb-clover mix, predicted ME of herb-clover mix with the ME of plantain and chicory stems included ($PME_{with\text{-}stems}$) and without $PME_{no\text{-}stems}$ during November 2016 to May 2017.

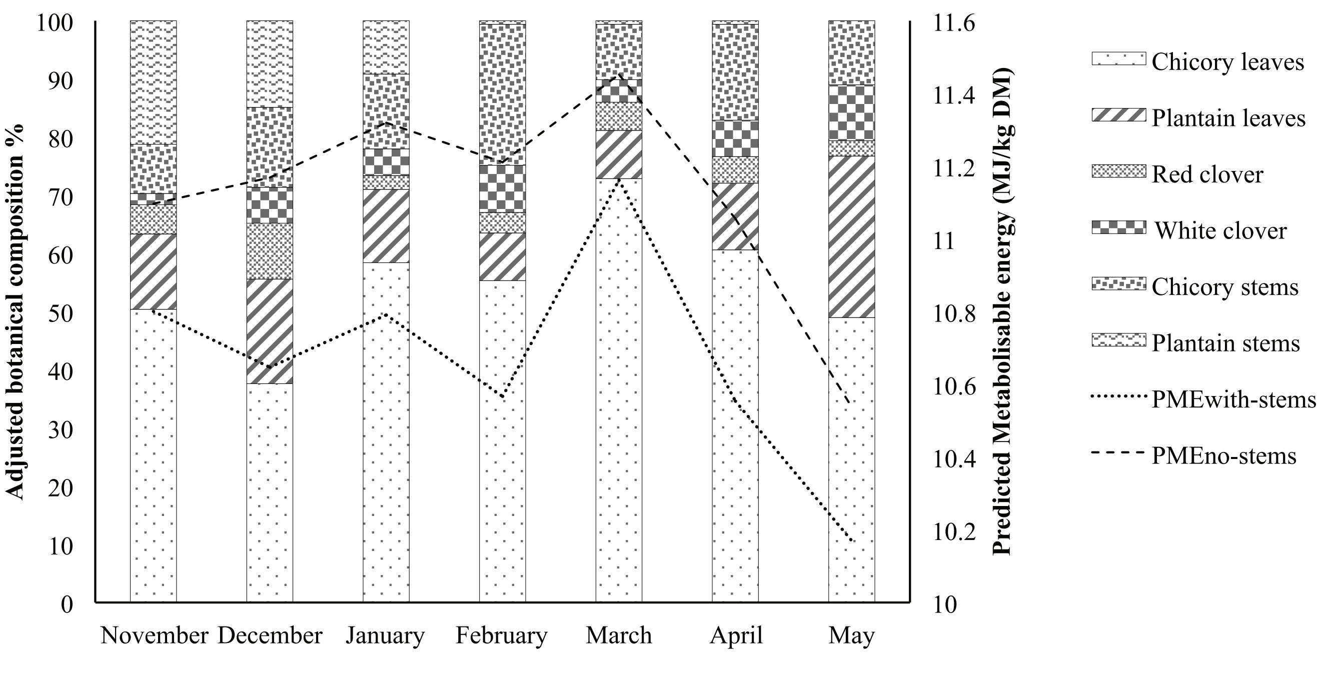

Table 2 Acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin of plant components in the herb-clover mix during November 2016 to May 2017 (Mean ± SEM).

Plant species/ component	November	December	January	February	March	April	May	SEM
	ADF (%)							
Chicory leaf	18.3^{blmn}	15.6^{ablm}	17.1^{abl}	16.0^{abl}	13.1^{al}	16.9^{abl}	19.9^{bl}	1.7
Chicory stem	23.6^{an}	38.5^{bn}	45.6^{cn}	46.5^{cn}	46.8^{cn}	60.1^{dn}	66.3^{en}	1.7
Plantain leaf	20.4^{ablmn}	19.8^{ab}	18.4^{al}	19.8^{abl}	16.3^{al}	18.4^{al}	24.3^{bm}	1.7
Plantain stem	30.7^{ao}	40.9^{bn}	40.1^{bm}	38.7^{bm}	30.8^{am}	40.9^{bm}	-	1.7
Red clover	15.4^{abl}	14.4^{al}	19.5^{bcl}	20.9^{cl}	15.5^{abl}	17.5^{abcl}	21.3^{clm}	1.7
White clover	16.5^{ablm}	17.4^{ablm}	17.5^{abl}	17.4^{abl}	14.1^{al}	15.8^{abl}	19.0^{bl}	1.7
	NDF (%)							
Chicory leaf	22.9^{bl}	19.2^{abl}	18.0^{abl}	19.0^{abl}	16.6^{al}	21.0^{abl}	22.9^{abl}	1.9
Chicory stem	30.4^{al}	48.4^{bm}	55.8^{co}	56.9^{co}	57.6^{co}	66.8^{dn}	76.8^{em}	1.9
Plantain leaf	25.9^{abl}	25.7^{abl}	23.6^{abm}	24.7^{ablm}	20.8^{am}	22.1^{al}	28.4^{bl}	1.9
Plantain stem	46.1^{am}	53.3^{bm}	53.2^{bo}	49.0^{bn}	43.7^{an}	54.0^{bm}	-	1.9
Red clover	24.9^{abcl}	21.4^{al}	27.1^{bcdn}	30.3^{dm}	22.8^{abm}	24.7^{abcl}	29.1^{cdl}	1.9
White clover	22.0^{abl}	23.0^{abl}	22.5^{abm}	21.3^{abl}	19.5^{alm}	20.6^{al}	26.5^{bl}	1.9

Note: SEM, standard error of the mean. Red clover and white clover were not separated in to leaves and stems.

$^{a\text{-}f}$ Means with different superscripts within rows are significantly different across months (P<0.05).

$^{l\text{-}p}$ Means with different superscripts within columns are significantly different across plant components (P<0.05).

Discussion

The aim of this study was to characterise the nutritional composition of individual plant components of the herb-clover mix during November to May period. In general, ME and CP of chicory and plantain leaves, red clover and white clover were higher than those of chicory and plantain stems during November to May period, while the NDF and ADF of plantain and chicory stems were higher than that of other plant components. Combined, this suggests that a farmer should manage the sward to allow for a higher proportion of chicory and plantain leaves, red clover and white clover during November to May to maximise the ME and CP of a herb-clover mix. The recommendations provided by Lee et al. (2015) to maximise leaf growth of plantain and chicory in pure swards could be applied to herb-clover mix. Maximised leaf growth would also reduce the overall NDF and ADF of a herb-clover mix. Previous studies have shown that lambs demonstrate preferences for plantain and chicory leaves and clover (Cave et al. 2015; Pain et al. 2010) and show an aversion to reproductive stems in the herb-clover mix (Fraser and Rowarth 1996). By having a higher proportion of leaves and clover in the herb-clover mix, therefore, improved animal performance should be achieved. Stems of both chicory and plantain can accumulate in the herb-clover mix greatly during summer than in late spring (Fraser and Rowarth 1996; Rumball 1986) resulting in higher ADF and NDF which reduces feed intake and rumen outflow (Hodgson and Brookes (1999) and thus, animal performance. Further, Hodgson and Brookes (1999) reported that the higher concentrations of ADF and NDF in herbages can negatively affect CP and ME concentrations, further reducing animal performance. Somasiri (2014) and Cave et al. (2015) reported that a herb-clover mix contained lower ME and CP and higher ADF and NDF during summer than in early spring. This was likely due to the higher proportions of plantain and chicory stems, which are high in ADF and NDF in summer than in early spring. The percentage of chicory and plantain stems in the herb-clover mix in this study was higher in early spring and summer than in autumn. The predicted ME of herb-clover mix, without ME of chicory and plantain stems, was also higher in late spring and summer than in autumn. Therefore, these results further suggest that the proportions of chicory and plantain stems greatly influence the overall ME of the herb-clover mix.

In previous studies, ME and CP of the herb-clover mix ranged from 9.8 to 11.6 MJ/kg DM and 9 to 25% DM, respectively, based on whole herb-clover samples (Kenyon et al. 2010; Cranston 2014). However, it is known that animals prefer different plant components in the mix (Cave et al 2015; Pain et al 2010; Fraser and Rowarth 1996). This suggests that measuring ME and CP of a whole mixed herb-clover samples may not actually represent what an animal consume. Therefore, using mixed herbage samples in analysis limits the ability to explain any varaiation in animal performance using these values. The results of the present study suggest that future nutritional analysis of the herb-clover mix should consider which plant components animals have eaten and their relative propotions in the mix, in order to be able to better explain animal performances on herb-clover mix.

Conclusion

Nutritional and botanical composition of individual plant components of the herb-clover mix changed during during November to May period. Metabolisable energy and CP of chicory leaves, plantain leaves, red clover and white clover were higher than that of chicory and plantain stems during November to May period. NDF and ADF of plantain and chicory stems were higher than that of other plant components. Overall nutritional value of the herb-clover mix was likely to be driven by the proportion of chicory and plantain stems. Therefore, it is important to keep the growth of chicory and plantain stems under control for higher CP and ME of herb-clover mix during November to May.

Acknowledgement

The authors thank Beef+Lamb New Zealand and Massey University for the financial support, and D Burnham and Keeble farm staff for technical assistance.

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