EVALUATION OF HERB PASTURES FOR NEW ZEALAND DAIRY SYSTEMS

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Soledad Del Carmen Navarrete Quijada

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I dedicate this Thesis to the memory of my wonderful grandma,

Mi abuelita Mary (1914 – 2015)

Thanks for your love and live so long for us

ABSTRACT

Chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) sown in pure swards or both herbs sown with red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.) are able to produce large amounts of high-quality forage in summer and autumn, when production and quality of perennial ryegrass (*Lolium perenne* L.) are often limited. There have been many cases of poor persistence of chicory with current management practices, but there is limited information for plantain and the herb-clover mix pastures when grazed in dairy systems. Additionally, the presence of bioactive compounds in plantain has created interest in their potential effect on rumen fermentation. The objectives of this research were to evaluate the effect of grazing frequency, two and four weeks, on the agronomic characteristics and diet selection of chicory, plantain, and herb-clover mix pastures, with dairy cows during two growing seasons (2011-2012; 2012-2013) and to examine the effect of the bioactive compounds in plantain on *in vitro* rumen fermentation.

Chicory plants grazed every two weeks, rather than every four weeks, did not develop large taproots, and consequently stored less carbohydrate reserves (fructan and fructose) and failed to develop extra shoots. Chicory grazed every two weeks failed to persist, while chicory grazed every four weeks persisted only for the two growing seasons. For plantain the grazing frequency did not affect its plant density nor taproot diameter; however, the number of shoots per plant increased when grazed every two weeks compared to every four weeks. Overall, plantain and herb-clover mix pastures were more productive and persistent than chicory. The three pastures produced high-quality feed for dairy cows. Dairy cows preferred the herb-clover mix pasture, rather than pure chicory or plantain swards, and selected indiscriminately between chicory, plantain, and red clover. The diet selection for chicory and plantain varied between seasons and was affected by the vertical access and horizontal distribution of the species in the swards. The presence of bioactive compounds, aucubin and acteoside in plantain reduced the ammonia concentration during fermentation and probably causes less urea to be excreted in the cow's urine when grazing plantain. In conclusion, the herb-clover mix performed best due to its high herbage production and nutritive value and greater grazing preference by dairy cows, plus its potential to reduce the nitrogen lost from dairy systems.

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LIST OF ABBREVIATIONS

A	Potential of gas production
Ac	Acteoside
ADF	Acid detergent fibre
AM	Morning
Au	Aucubin
BCVFA	Branched chain volatile fatty acids
CH	Chicory
CH+10au	Chicory plus 10 mg aucubin/g DM
CH+20au	Chicory plus 20 mg aucubin/g DM
CH+40ac	Chicory plus 40 mg acteoside/g DM
CO_2	Carbon dioxide
CP	Crude protein
CT	Condensed tannins
DM	Dry matter
DMI	Dry matter intake
ELH	Extended leaf height
FV	Feeding value
GIS	Geographic Information System
GP	Gas production
GPS	Global Positioning System
HPLC	High-performed liquid chromatography
HWSC	Hot water soluble carbohydrates
LAI	Leaf area index
LTA	Long term average
LW	Live weight

ME	Metabolisable energy
MeOH	Methanol
N	Nitrogen
N2O	Nitrous oxide
NDF	Neutral detergent fibre
NH_3	Ammonia
OM	Organic matter
OMD	Organic matter digestibility
PL	Plantain
PL+10au	Plantain plus 10 mg aucubin/g DM
PL+36ac	Plantain plus 36 mg acteoside/g DM
PM	Afternoon
$R^{1/2A}$	Fermentation rate at T1/2A
RA	Relative abundance
RFC	Readily fermentable carbohydrates
SC	Structural carbohydrate
Si	Selection index
$T^{1/2A}$	The half time when the potential gas production was reached
V24h	Volume of gas produced after 24 h incubation
VFA	Volatile fatty acid
VFI	Voluntary feed intake
WSC	Water soluble carbohydrate

1 GENERAL INTRODUCTION

Pasture based dairy systems in New Zealand need to maintain a feed supply to meet the feed demands of cows. This system has traditionally been based on the herbage production of perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) pastures (Charlton and Stewart, 1999). The increasing incidence of dry summers in recent years and the absence of irrigation on most dairy farms can seriously limit the growth of perennial ryegrass/white clover pasture leading to greater feed deficits and lower milk production from summer to autumn (Kemp et al., 2010; Lee et al., 2013). Under these circumstances, the use of alternative forage species with greater drought tolerance, such as chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.), is now common on many dairy farms to supplement dairy cows during summer and autumn (Glassey et al., 2013; Lee et al., 2015).

Chicory and plantain provide herbage of high nutritive value, with their greatest herbage mass accumulated during late spring and summer (Lee et al., 2015; Li and Kemp, 2005; Powell et al., 2007). Under New Zealand dryland conditions, annual dry matter (DM) production of up to 15 t DM/ha has been obtained for chicory (Brown et al., 2003) and between 15-19 t DM/ha for plantain (Minnee et al., 2013; Powell et al., 2007). An agronomic concern with chicory and plantain is their pasture production and persistence under grazing management with dairy cows. The ability of chicory and plantain to maintain high yielding pastures over time is largely influenced by their plant density and loss of plants is the major factor affecting pasture persistence (Neal et al., 2009; Tozer et al., 2011). Chicory has been reported to persist for three years under sheep and deer grazing (Li and Kemp, 2005), but anecdotal evidence suggested that chicory persists for only one year under current widely used management on dairy farms. Plantain reportedly has a greater persistence than chicory (Glassey et al., 2013; Powell et al., 2007); however, there is still limited information on plantain grazing management on dairy farms.

Both herbs produce herbage of high quality for ruminants during summer and autumn (Barry, 1998; Kemp et al., 2010). These pastures have been shown to improve milk solids production in dairy cows when included in the diet with perennial ryegrass/white clover pastures during summer and autumn compared to those fed solely perennial ryegrass/white clover (Minnee et al., 2012; Waugh et al., 1998). This is due to greater dry matter intake (DMI) of a forage with higher nutritive value per unit of DM eaten (Barry, 1998). However, diet selection of animals affects their DMI and under grazing conditions sward characteristics, such as availability and accessibility, are constraints that influence DMI (Hodgson, 1985).

The use of multi species pastures, sowing more than four species, was initially proposed to increase DM production during late spring and summer compared to perennial ryegrass/white clover (Sanderson et al., 2005). Nevertheless, Kemp et al. (2010) suggested that combining chicory, plantain, red clover (*Trifolium pratense* L.), and white clover, in a herb-clover mix, could also provide greater DM production of high quality that increased the length of the growing season, particularly under dryland conditions. Herb-clover mix pastures have been evaluated with sheep, showing greater ewe milk production, higher lamb weaning weights, and greater lamb growth rates post-weaning when compared to perennial ryegrass (Golding et al., 2011; Hutton et al., 2011; Kemp et al., 2010; Kenyon et al., 2010). Research on mixed pastures that included plantain has shown reduced excretion of N in the urine of cows (Totty et al., 2013; Woodward et al., 2013). Plantain contains biologically active secondary compounds, catalpol, aucubin, and acteoside (Stewart, 1996; Tamura and Nishibe, 2002). These compounds have antimicrobial activity (Andary et al., 1982; Davini et al., 1986; Kim et al., 2000), which may affect rumen function and are likely to have important implications for rumen nitrogen (N) efficiency of grazing animals (Stewart, 1996); but, the extent of their impact on rumen fermentation is unclear. These findings suggest that plantain and herb-clover mix pastures may have the potential to improve milk production in dairy systems with the added benefit of improved N use efficiency of cows and reduced N leaching into the environment compared to chicory. However, there is limited information on their grazing management, pasture persistence and nutritive value when grazed with dairy cows.

1.1 THESIS OBJECTIVES

Therefore, the general objectives of this thesis are:

I. To examine the agronomic characteristics, such as herbage production, pasture persistence, plant development, and nutritive value of chicory and plantain, as pure swards and in herb-clover mix pastures.

II. To determine the diet selection and grazing preference of dairy cows for chicory, plantain, and herb-clover mix pastures.

III. To investigate the effect of the bioactive compounds in plantain on *in vitro* rumen fermentation.

1.2 THESIS STRUCTURE

This thesis is presented in a series of seven chapters. This general introduction (Chapter 1) states the reasons and importance of this research. The literature review (Chapter 2) provides an overview of the current knowledge on herb pastures based on previous findings and identifies the gaps that provide the focus for the experimental work. The research chapters (Chapters 3 to 6) are presented as papers, which can be readily prepared for publication, and results are discussed in detail in each research chapter. Finally, an overall discussion integrates the key findings from this research, and presents the main conclusions, including potential for future research (Chapter 7).

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2 LITERATURE REVIEW

2.1 DESCRIPTION AND HISTORY OF CHICORY AND PLANTAIN

Chicory (*Cichorium intybus* L.) and plantain *(Plantago lanceolata* L.) are perennial herb species native to Europe (Foster, 1988; Ivins, 1952; Rumball, 1986). Both species were considered weeds; chicory was commonly found along roadsides and in waste areas (Rumball, 1986) and plantain occurred naturally in grasslands and was broadly distributed throughout the temperate world (Foster, 1988). Historically, both chicory and plantain have been cultivated for multiple uses. Both herbs are grown as medicinal plants with attributes such as anthelmintic properties (Knight et al., 1996; Rumball, 1986). Chicory is also cultivated as a vegetable crop where its leaves and shoots are used for salads and vegetables dishes, and its roots can be used as a coffee substitute and additive. Chicory root is also used as a source of inulin to produce fructose (Wang and Cui, 2013). The use of chicory and plantain as livestock forage is more recent with cultivar development carried out in New Zealand.

Chicory is a rosette plant, has broad leaves, and a long thick taproot (Labreveux et al., 2004). The leaves of chicory are produced from the crown, which in spring produces large leaves in response to warm temperatures (Rumball, 1986). Plantain is also described as a rosette plant with simple leaves and a short taproot followed by deep fibrous roots (Foster, 1988; Ivins, 1952). Plantain leaves are lanceolate to oval lanceolate 3 to 7 cm in length with parallel veins and a petiole about half the length of the lamina (Rumball et al., 1997). Reproductive stems in plantain emerge from the centre of the rosette forming cylindrical flowerheads at the end of the stem (Sagar and Harper, 1964).

2.1.1 Breeding of the herbs for pastoral grazing systems in New Zealand

2.1.1.1 Chicory

The interest in chicory as livestock forage began in the late 1970s'. Lancashire (1978) reported chicory as having excellent nutritive value under rotational grazing. The large variation between plants in characteristics such as yield, longevity, disease resistance, and flower and seed production indicated that it might be possible to select material that would be suitable for New Zealand dryland farming regions (Rumball, 1986). Cultivar development began in 1975 in Palmerston North, New Zealand.

Chicory was selected for pasture production under New Zealand conditions and after more than 10 years of selection, the cultivar ‘Grassland Puna’ became commercially available in 1985 as the world’s first chicory forage cultivar (Rumball, 1986). Puna chicory was selected for vigorous uniform plants with dense leaf growth (Rumball, 1986). This cultivar showed high growth rates during late spring and summer (Hare et al., 1987), producing a large number of erect leaves (Rumball, 1986), with higher levels of potassium (K), sodium (Na), calcium (Ca), sulphur (S), boron (B), manganese (Mn), and zinc (Zn) than perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (Crush and Evans, 1990). Puna chicory was readily grazed by animals, promoted high animals growth rates and had no harmful side effects (Barry, 1998). This resulted in Puna chicory being widely adopted by farmers for use as grazing forage. However, Puna chicory fed as a sole diet to dairy cows can produce a bitter aftertaste in milk products, due to the sesquiterpene lactones present in the chicory leaves (Price et al., 1990). For this reason, it was recommended to limit the feeding of Puna chicory to dairy cows to only 2 h/d to restrict chicory intake to approximately 25% of their total daily dry matter (DM) intake (Barry, 1998).

The chicory cultivar 'Grassland Choice' was selected for lower levels of sesquiterpene lactones and for greater winter growth, as well as uniformity in plant morphology, thus being less likely to cause problems related to milk taint on dairy farms (Rumball et al., 2003b). Another cultivar of chicory, 'Puna II', was selected for higher levels of sesquiterpene lactones, lactucin and lactucopicrin to improve tolerance to the fungus *Sclerotinia*, cool-season activity, and more uniform plants (Rumball et al., 2003b). Both, Choice and Puna II chicory, were bred from the cultivar 'Grassland Puna'. The Choice chicory was targeted for dairy farming systems, whereas the Puna II chicory was intended as a general purpose replacement for Puna chicory on non-milking farms as a pure sward or as a component of mixed swards on dairy farms (Rumball et al., 2003b).

Two other forage cultivars of chicory are 'Lacerta' and 'Forage Feast'. Lacerta chicory is a synthetic variety derived from an ecotype grown by Uruguayan farmers. It is more uniform and has a more erect growth habit, but a lower proportion of plants remain vegetative in the first year compared with Puna chicory (Foster et al., 2002). Forage Feast chicory is derived from a specialist group of root chicory varieties used for inulin production and was selected for uniformity in vegetation and time of bolting in France (Foster et al., 2002). Puna, Puna II, Choice, Grouse and Chico are some of the chicory cultivars now available in New Zealand (Li and Kemp, 2005).

2.1.1.2 Plantain

Early research reported that plantain was a palatable pasture plant for grazing animals such as sheep (Milton, 1933). Plantain is a good source of minerals; Ca, K, Na, chlorine (Cl), phosphorus (P), and cobalt (Co) (Sagar and Harper, 1964). Plantain was recommended as a constituent of pasture mixtures because it has been shown to improve animal health (Thomas et al., 1952). These findings opened the possibility that plantain could also be used as a pasture species. Cultivar development of plantain began in 1987 in New Zealand (Rumball et al., 1997).

The first two cultivars of plantain for grazing systems were ‘Grassland Lancelot’ and ‘Ceres Tonic’. Grassland Lancelot was the first plantain cultivar released for pasture use in New Zealand (Rumball et al., 1997). This cultivar was selected from a widespread collection of plants obtained in March 1987 within the Manawatu Region of the North Island in New Zealand (Stewart, 1996). The aim was to identify plants with high seed production and vegetative yield (Rumball et al., 1997). Grassland Lancelot was selected for a bushy growth habit and the ability to tiller strongly under close sheep grazing; however, it may become prostrate under these conditions (Stewart, 1996). This cultivar is described as a perennial plant with a rosette form, more uniform growth, more erect and with a higher number of vegetative shoots, compared with the naturalized genepool from which it was developed (Rumball et al., 1997).

The plantain cultivar ‘Ceres Tonic’ was selected for a more erect habit and large leaves (Stewart, 1996). It was selected from germplasm derived from northern Portugal, a region with warmer winters than most of New Zealand (Stewart, 1996). Tonic plantain remains erect under a wide range of grazing management (Stewart, 1996). This cultivar is considered to be very different from Lancelot, with large leaves, more erect growth, and greater winter activity (Rumball et al., 1997; Stewart, 1996). Lancelot plantain has now been withdrawn from the market due to the superiority of Tonic plantain, which is now used in many temperate regions around the world, including the USA, European countries, Uruguay, Chile, Australia and New Zealand.

2.2 AGRONOMIC FEATURES

2.2.1 Establishment

Chicory and plantain establish and grow in a wide range of soil types. Chicory grows best on well-drained soils of medium to high fertility (Hare et al., 1987) and within a normal range of soil acidity (pH 5.6 - 6.5) (Moloney and Milne, 1993). Plantain, however, adapts to soils of different textures and organic matter levels (Mook et al., 1989), tolerates a higher range of soil acidity (pH 4.2 - 7.8) (Stewart, 1996) and lower fertility conditions (Olff and Bakker, 1991; Troelstra et al., 1992). Plantain is, in particular, tolerant of low levels of K and P (Cavers et al., 1980; Stewart, 1996).

Glassey et al. (2013) recommended that chicory and plantain should be established by applying herbicide to existing vegetation and direct drilling seed. Weeds should be eliminated before sowing due to the lack of herbicide that can be applied post emergence (Glassey et al., 2013). Both herbs are usually sown at high rates; typically 6 - 8 kg seed/ha (Glassey et al., 2013; Lee et al., 2015; Li and Kemp, 2005; Powell et al., 2007). The sowing rate is based on the weight of 1000 seeds (approximately 1.5 g for chicory and plantain) (Rumball, 1986; Stewart, 1996) and aims to establish adequate plant numbers per unit area (Powell et al., 2007) to ensure the potential for high yield pastures (Hume et al., 1995).

Pastures of chicory and plantain can be established in autumn or spring. Autumn sowing is widely used in New Zealand to establish new pastures and perennial forage crops so that new plants are established and grazed before winter (Powell et al., 2007). Chicory when sown in early autumn is less competitive (Moloney and Milne, 1993) and is ready for first grazing in August (Powell et al., 2007). Chicory establishes best from mid-spring sowings, with the first grazing easily possible by December (Hume et al., 1995). Plantain establishes well either when sown in early autumn or spring, and is typically ready for first grazing before chicory (Powell et al., 2007). In fact, plantain emergence from autumn sowing is rapid and faster than many common grasses (Stewart, 1996).

2.2.2 Growth and herbage production

Early evaluations in New Zealand reported that the yield of chicory was up to 8.5 t DM/ha (Rumball, 1986) and for plantain up to 20 t DM/ha (Stewart, 1996). However, it was later shown that chicory was capable of greater yields in New Zealand conditions with chicory being able to produce between 15 - 18 t DM/ha, and up to 25 t DM/ha (Hare et al., 1987; Matthews et al., 1990). Both chicory and plantain accumulate their greatest herbage mass during late spring and summer due to their drought tolerance (Lee et al., 2015; Li et al., 1997a; Powell et al., 2007). Plantain has greater herbage accumulation than chicory, except during summer, when both herbs grow at similar rate due to their heat tolerance (Powell et al., 2007).

Table 2-1 presents the production of chicory and plantain obtained in several trials in New Zealand. Under New Zealand grazing management, the annual production of pure chicory swards ranged between 9 - 14 t DM/ha (Li and Kemp, 2005; Powell et al., 2007), and between 10 - 17 t DM/ha for plantain (Lee et al., 2015; Powell et al., 2007). In New Zealand, under dryland conditions, the average yield over a five year period for chicory has been 16 t DM/ha (Brown et al., 2003). In contrast, under irrigation, the potential annual production for chicory ranges from 13 - 20 t DM/ha, as was demonstrated over a six year period in Canterbury, New Zealand (Brown and Moot, 2004).

Table 2-1 Herbage production (t DM/ha) obtained for chicory and plantain, in New Zealand.

Period	Chicory	Plantain	References
1994/1995 (Nov - Apr)	5-9		Li et al. (1997a)
1997/1998	16		Brown et al. (2003)
2001/2002	11		Brown et al. (2003)
1997/1998 (Jul - Jun)	19*		Brown and Moot (2004)
2001/2002 (Jul - Jun)	15		Brown and Moot (2004)
2006/2007 (Jul - Mar)	14	17	Powell et al. (2007)
2010/2011 (Dec - May)	8 - 11	11 - 16	Lee et al. (2015)
2011/2012 (Aug - May)	10 - 11	10 - 14	Lee et al. (2015)
2009/2010 (Nov - Jun)	9 - 11	10 - 12	Glassey et al. (2013)
2011 (Jan - Apr)	6		Tozer et al. (2012)

***** grown under irrigation

2.3 NUTRITIVE VALUE

The nutritive value of both chicory and plantain has been thoroughly evaluated in a wide range of trials. The nutritive value of a forage is a measure of available nutrients with the important nutritive traits being: organic matter digestibility (OMD); metabolisable energy (ME); crude protein (CP); water soluble carbohydrates (WSC) and neutral detergent fibre (NDF) concentrations (Waghorn and Clark, 2004). Chicory often has a greater CP concentration and OMD compared to perennial ryegrass (Barry, 1998), whilst the opposite has been reported for plantain (Li and Kemp, 2005; Swainson and Hoskin, 2006). Both herbs are higher in WSC and lower in NDF concentrations than perennial ryegrass (Fraser and Rowarth, 1996). As a result, higher ratio of readily fermentable carbohydrates (RFC) to structural carbohydrates (SC) is obtained with these herbs than with perennial ryegrass (Barry, 1998; Burke et al., 2000). Table 2-2 shows the CP, NDF and OMD reported for chicory and plantain, respectively.

Table 2-2 Seasonal concentration (g/kg DM) of crude protein (CP), neutral detergent fibre (NDF), and organic matter digestibility (OMD) for chicory and plantain, in New Zealand.

	Chicory			Plantain			
Season	CP	NDF	OMD	CP	NDF	OMD	References
Spring	218	-	853	-	-	-	Min et al. (1997)
	-	-	-	120	380	750	Swainson and Hoskin (2006)
Late spring	147	349	726	-	-	-	Komolong et al. (1994)
	197	168	897	175	231	859	Jackson et al. (1996)
Summer	240	170	848	202	223	804	Fraser and Rowarth (1996)
	147	255	834	-	-	-	Waugh et al. (1998)
	206	222	779	204	280	720	Gregorini et al. (2013)
	250-280	270	-	160-280	300-390	650-700	Lee et al. (2015)
	-	-	-	160-200	320-340	740-760	Cave et al. (2015)
Autumn	218	-	820	-	-	-	Min et al. (1997)
	172	198	764	-	-	-	Waugh et al. (1998)
	-	-	-	250	280	850	Burke et al. (2000)
	200	270	-	200-280	220-250	750	Lee et al. (2015)

2.3.1 Protein

Chicory has a higher concentration of CP than plantain in spring, summer, and autumn (Table 2-2). Levels of CP in chicory fluctuate from 147 - 280 g/kg DM (Komolong, 1994; Labreveux et al., 2006; Lee et al., 2015; Sanderson et al., 2003), with a mean CP concentration ranging between 180 - 200 g/kg DM (Brown and Moot, 2004; Holden et al., 2000; Jung et al., 1996; Volesky, 1996). The concentration of CP in chicory is lower than in legumes, but higher than perennial ryegrass (Li and Kemp, 2005). In New Zealand, chicory has a greater CP concentration than perennial ryegrass, with levels exceeding 200 g/kg DM during spring (Min et al., 1997; Sun et al., 2011) and summer (Fraser and Rowarth, 1996; Kusmartono et al., 1997; Minnee et al., 2012; Waugh et al., 1998). In contrast, the concentration of CP in autumn has been reported to be greater in perennial ryegrass than chicory (Burke et al., 2000; Sun et al., 2012; Waugh et al., 1998).

The CP concentration of plantain has ranged from 120 - 280 g/kg DM (Swainson and Hoskin, 2006; Lee et al., 2015). During spring and summer plantain, has a CP concentration lower than perennial ryegrass (Hoskin et al., 2006; Minnee et al., 2012; Swainson and Hoskin, 2006). However, CP concentration in plantain leaf during summer has been reported to be similar to perennial ryegrass when the stem component was removed (Burke et al., 2000; Fraser and Rowarth, 1996). The lowest CP concentration of plantain is obtained in summer, ranging between 105 - 130 g/kg DM (Cave et al., 2015; Lee et al., 2015; Sanderson et al., 2003). The lower CP concentration of plantain during summer has been associated with a greater proportion of stems in swards, as CP concentration of the stem is lower than the leaf (Lee et al., 2015). The CP concentration decline was greater when plantain was under soil moisture stress during summer (Kemp et al., 2014). During autumn, the CP concentration of plantain has shown to be similar to perennial ryegrass (Burke et al., 2000; Hoskin et al., 2006).

2.3.2 Fibre

Both herbs contain less fibre than perennial ryegrass (Barry, 1998; Burke et al., 2000; Fraser and Rowarth, 1996; Kusmartono et al., 1997; Swainson and Hoskin, 2006). Lignin and pectin are higher in both herbs, whereas cellulose and hemicellulose are lower when compared to perennial ryegrass (Barry, 1998; Kusmartono et al., 1997; Swainson and Hoskin, 2006). Chicory has lower NDF, hemicellulose and lignin, but similar cellulose concentrations compared to plantain leaf (Fraser and Rowarth, 1996).

NDF concentration has ranged from 170 - 380 g/kg DM for chicory and from 220 - 390 g/kg DM for plantain (Table 2-2). Chicory has lower NDF concentrations during the first growing season compared to the subsequent growing seasons (Holden et al., 2000; Labreveux et al., 2006). During the first year, spring sown chicory is in a vegetative stage and has low stem production (Lee et al., 2015; Li and Kemp, 2005). Chicory develops stems in the following spring as this is triggered by vernalisation during winter, producing flowers and seed from its second growing season onwards and increasing the NDF concentration (Moloney and Milne, 1993). Despite the rapid stem growth in summer, chicory NDF concentration has not been reported to have exceeded 300 g/kg DM (Table 2-2).

Plantain NDF concentration is greater than chicory (Table 2-2). The higher NDF is observed during summer, with concentrations ranging from 280 - 390 g/kg DM (Cave et al., 2015; Lee et al., 2015; Minnee et al., 2012). Stem production in plantain increases in late spring and summer (Fraser and Rowarth, 1996), with the stems containing a higher NDF concentration than the leaves (Fraser and Rowarth, 1996; Jackson et al., 1996; Lee et al., 2015). Plantain stems in the sward increased NDF concentration and also decreased CP concentration (as discussed in section 2.3.1) affecting nutritive value during summer.

2.3.3 Organic matter digestibility

There are seasonal differences in OMD between chicory and plantain, and perennial ryegrass. Perennial ryegrass has an OMD that peaks in spring (850 g/kg DM), declines in summer (700 g/kg DM) and then increases in autumn to 780 g/kg DM (Kusmartono et al., 1996a). However, chicory in the vegetative state maintains a more stable OMD (approximately 850 g/kg DM) throughout the seasons (Barry, 1998), which is greater than both perennial ryegrass (Barry, 1998; Komolong, 1994; Kusmartono et al., 1996a) and plantain (Fraser and Rowarth, 1996; Labreveux et al., 2006; Lee et al., 2015; Sanderson et al., 2003). Sanderson et al. (2003) reported an OMD 11% greater in chicory than plantain in both spring and summer, with an average value of 850 and 765 g/kg DM for chicory and plantain, respectively.

The OMD of plantain tends to fluctuate over the growing season, with levels as high as perennial ryegrass (Stewart, 1996). The OMD obtained for plantain during spring and summer has ranged from 650 - 780 g/kg DM (Table 2-2). During spring, the OMD of plantain is lower than perennial ryegrass (Hoskin et al., 2006; Swainson and Hoskin, 2006), but greater than perennial ryegrass during autumn (Burke et al., 2000). Plantain leaves have similar OMD (approximately 800 - 850 g/kg DM) as chicory during late spring and summer (Fraser and Rowarth, 1996; Jackson et al., 1996), and an OMD similar to perennial ryegrass when the reproductive stem was removed, from spring harvest (Stewart, 1996). The high propensity of plantain to develop reproductive stems negatively affects its digestibility (Sanderson et al., 2003) as well as its CP and NDF concentration (as discussed in sections 2.3.1 and 2.3.2), with a bigger impact in summer when stems can make up 60% of the plantain on offer (Fraser and Rowarth, 1996). This demonstrates the importance of controlling stem development in plantain to maintain high nutritive value.

The higher OMD in both herbs is attributed to the high RFC:SC ratio, reported as being three times greater than perennial ryegrass (Kusmartono et al., 1997; Swainson and Hoskin, 2006). This high RFC:SC ratio results in both herbs disintegrating rapidly in the rumen with DM losses of 0.26% and 0.25% per hour for chicory and plantain, respectively (Barry, 1998; Burke et al., 2000). The faster rumen degradation of chicory and plantain reduces their mean retention time, increasing rumen clearance, and hence allowing greater voluntary feed intake (VFI) in animals (Kusmartono et al., 1996a).

In contrast, the NDF in chicory and plantain has lower digestibility, mainly due to the hermicellulose fraction, compared to perennial ryegrass (Barry, 1998; Swainson and Hoskin, 2006). Kusmartono et al. (1997) commented that the higher degradation rate of chicory with a lower mean retention time may allow less time for microbial digestion. Furthermore, the high RFC:SC may produce a lower rumen pH for chicory fed animals and affect the activity of rumen microorganisms degrading cellulose and hemicellulose. Barry (1998) reported a lower pH in deer grazing chicory (pH 5.7) than those grazing ryegrass (pH 6.5) with the low pH likely to affect the hemicellulose fermentation by rumen microorganisms.

2.3.4 Secondary compounds

Secondary compounds are organic compounds synthesized by many plants that are not strictly essential to the main function of the plants, such as their growth and reproduction, but rather associated with plant defence mechanisms against pathogens and herbivores (Acamovic and Brooker, 2005; Athanasiadou and Kyriazakis, 2004). However, secondary compounds can exert both anti-nutritional and nutritional effects upon the forage nutritive value (Barry et al., 2002). A vast array of chemical compounds and concentrations can occur in forage species (Jackson et al., 1996). The secondary compounds that occur in chicory and plantain are summarised in Table 2-3.

Table 2-3 Secondary compounds present in chicory and plantain.

Species	Total condensed tannin content (g/kg DM)	Other known plant secondary compounds
Chicory	4.2	Sesquiterpene lactones - Lactucin - Lactucopicrin - 8-deoxilactucin
Plantain	14	Iridoid glycosides - Catalpol - Aucubin Phenilpropanoide glycose - Acteoside

Adapted from (Barry et al., 2002)

The secondary compounds most extensively studied have been condensed tannins (CT) and their effect on animal nutrition due to the ability to protect protein from rumen degradation. Chicory and plantain are considered to have a low and medium levels of CT, respectively (Barry, 1998; Stewart, 1996). Chicory in the vegetative state has been shown to have a range of values between 0.5 - 2.5 g/kg DM (Kusmartono et al., 1996b); however, CT concentrations of 4.2 g/kg DM has also been observed in chicory (Barry et al., 2002). Plantain was reported to have levels of CT between 4 - 10 g/kg DM (Stewart, 1996), and a CT level of 14.1 g/kg DM has been obtained from plantain leaves harvested in mid-summer (Jackson et al., 1996). Levels lower than 17 g/kg DM are considered unlikely to protect protein from rumen degradation (Barry, 1998; Deaker et al., 1994). However, chicory and plantain contain other secondary compounds of importance.

2.3.4.1 Chicory

Chicory contains the sesquiterpene lactones: lactucin; lactucopicrin; and 8-deoxylactucin which are part of the defensive chemistry of the plant, deterring consumption by insects (Barry, 1998) and providing resistance to fungal diseases such as *Sclerotinia* spp (Rumball et al., 2003b). Lactucopicrin and 8-deoxylactucin are the major compounds in chicory while lactucin is always present in relative minor amounts (Rees and Harborne, 1985). Total sesquiterpene lactone concentrations in chicory leaves are affected by cultivar and seasons of the year (Rumball et al., 2003a). The total sesquiterpene lactone concentration was reported to be lower in autumn than in late spring and early summer (Foster et al., 2011).

Research has shown that acceptance of chicory herbage by ruminants is inversely related to total sesquiterpene lactones concentration, in particular to the concentration of lactucopicrin (Foster et al., 2006). The bitterness in chicory is caused by lactucin in its leaves (Price et al., 1990) and the unpleasant aftertaste is produced by lactucopicrin (Rumball et al., 2003a). Ewe lambs grazing a herb-clover mix pasture containing Puna II chicory selected less chicory during late spring and summer than in spring and autumn (Cave et al., 2015). Mature leaves contain higher concentrations of sesquiterpene lactones, making the herbage bitter and which might explain the observations of low palatability (Belesky et al., 1999; Horadagoda et al., 2009).

Chicory feed in dairy farms can cause a bitter aftertaste in milk products (Price et al., 1990). Degradation products of the sesquiterpene lactones are identified as the taint compounds in the milk of Puna chicory fed cows (Barry, 1998). For this reason, Choice chicory was bred with lower levels of sesquiterpene lactones than Puna chicory to avoid milk taint problems on dairy farms (Rumball et al., 2003a). Choice chicory has average lactucin and lactucopicrin values, 85% of that found in Puna chicory, and it is, therefore, likely to be less persistent than Puna chicory swards (Rumball et al., 2003a).

2.3.4.2 Plantain

Plantain contains iridoid glucosides catalpol, aucubin and phenylpropanoid glycoside acteoside (Stewart, 1996). The concentration of these compounds in plantain is affected by cultivar, environmental factors and leaf age (Tamura and Nishibe, 2002). Catalpol is present in plantain natural ecotypes and in the cultivar ‘Grassland Lancelot’ but is absent in the cultivar ‘Ceres Tonic’ (Al-Mamun et al., 2008; Tamura and Nishibe, 2002). Aucubin and acteoside are always present in plantain, with aucubin concentrations of 17.8 mg/g DM and acteoside with concentrations up to 90 mg/g DM (Fajer et al., 1992). Lower levels of 3.2 mg/g DM for aucubin (Al-Mamun et al, 2008) and 19 mg/g DM for acteoside (Tamura and Nishibe, 2002) have been reported for the cultivar ‘Ceres Tonic’.

These compounds are biologically active. Acteoside is known for having a antioxidative activity and aucubin stimulates the removal of uric acid from tissues to the blood, as well as the excretion of uric acid from the kidneys (Tamura, 2002). Deaker et al. (1994) found that lambs grazing plantain had heavier kidneys with no apparent impairment of renal function compared to those grazing perennial ryegrass. Greater urine flows have been recorded by animals grazing plantain (Wilman and Derrick, 1994), consistent with the diuretic effect of iridoid compounds. These compounds are also reported to have antimicrobial and antifungal effects (Andary et al., 1982; Davini et al., 1986; Kim et al., 2000). Research has found a markedly reduced ammonia (NH_3) concentration in the rumen fluid of deer fed plantain compared to those fed pasture, and suggested that this might be attributable to the presence of secondary compounds in plantain (Swainson and Hoskin, 2006). Antimicrobial compounds capable of affecting the rumen fermentation process are likely to have important implications for rumen function and efficiency (Stewart, 1996).

2.4 ANIMAL PERFORMANCE

Pasture based systems relies on perennial ryegrass pastures in New Zealand. The performance of animal grazing forage is usually limited by energy intake because structural fibre can slow digestion and clearance from the rumen (Waghorn and Clark, 2004). The growth pattern for perennial ryegrass shows high herbage production and quality during spring, but quality and yield decline over the summer due to increasing plant maturity and soil moisture deficits (Waghorn and Barry, 1987). The increasing incidence of dry summers in recent years has resulted in the herbage production of perennial ryegrass becoming more limited during summer and autumn (Lee et al., 2013), leading to feed deficits and lower animal production (Li and Kemp, 2005). Animal requirements during summer are usually high, for example, lambs post weaning being capable of fast growth, and dairy cows in mid lactation. Inadequate feed supply relative to animal requirements is an inherent limitation of the seasonality of the pasture feed supply in relation to animal production (Barry, 1998), resulting in animal performance being below the maximum genetic potential (Komolong, 1994).

2.4.1 Feeding Value

Chicory and plantain are species considered to have a high feeding value (FV) for ruminants (Barry, 1998; Kemp et al., 2010). Animal production obtained from animals grazing forage under unrestricted conditions best represents forage FV (Ulyatt, 1973), as it is a function of both VFI and the nutritive value per unit of DM eaten (Barry et al., 2002; Waghorn and Clark, 2004). The FV of chicory and plantain has been evaluated under grazing conditions using lambs (Fraser and Rowarth, 1996; Komolong, 1994), deer (Hoskin et al., 2006; Kusmartono et al., 1996a; Min et al., 1997), and cows (Minnee et al., 2012; Waugh et al., 1998).

Table 2-4 shows the animal production for chicory, plantain, and herb-clover mix pastures in comparison to perennial ryegrass pasture obtained in lambs, deer, and cows in New Zealand. Lambs and deer grow faster on chicory (over 250 g LW/d) and plantain (average 200 g LW/d) than on perennial ryegrass pasture (120 - 175 g LW/d) over the summer and autumn period (Table 2-4). Hence, the FV of chicory and plantain is considered superior to perennial ryegrass during these seasons (Barry, 1998).

Both chicory and plantain are forage options that can mitigate the feed deficit and maintain, or even improve, milk production during summer (Minnee et al., 2012). Waugh et al. (1998) reported that cows grazing chicory as a supplement during the summer and autumn periods had increased milk production, and indicated that the greatest benefit from feeding chicory was seen when pasture allowance was low (15 kg DM/cow/d), for instance during a summer drought. When perennial ryegrass pasture has low quality (9.6 MJ/kg DM) supplementation with chicory and plantain (either 20 or 40%) improved dry matter intake (DMI) and cows produced 19% more milk and 17% more milk solids than those fed pasture only. In addition, a diet including up to 60% herbs did not affect DMI or milk production, meaning this approach that can be used during a feed deficit without compromising milk production (Minnee et al., 2012).

The use of herb-clover mix pastures also provided a higher FV than perennial ryegrass (Kemp et al., 2010). Recent research has focused on the evaluation of herb and legume mixtures as alternative pasture for sheep production (Golding et al., 2011; Hutton et al., 2011; Kenyon et al., 2010; Sinhadipathige et al., 2012; Somasiri et al., 2013). Kenyon et al. (2010) reported that a mixed herb and legume sward offered to ewes in late pregnancy and early lactation increased the early lamb live weight gain; improved lamb survival; and increased the total live weight of lambs weaned. The better performance of the lambs was associated with greater milk production in ewes (Hutton et al., 2011).

Table 2-4 Liveweight gains (LWG) of lambs and deer grazing herbs species in comparison to perennial ryegrass pastures and milk production (l/cow/d) of dairy cows supplemented with herb pastures.

	Chicory	Plantain	Herb-clovers	Ryegrass	Reference
Lamb LWG (g/d)					
Spring	-	-	206	175	Hutton et al. (2011)
Early spring	-	-	360	322	Somasiri et al. (2013)
Late spring	-	-	262	193	Somasiri et al. (2013)
Late spring	-	376	-	296	Judson et al. (2009)
Summer	280	-	-	158	Scales et al. (1995)
Summer	-	222	-	135	Moorhead et al. (2002)
Summer	-	-	214	169	Somasiri et al. (2013)
Summer to early autumn	192	164	-	121	Fraser and Rowarth (1996)
Deer LWG (g/d)					
Spring	333	-	-	292	Min et al. (1997)
Summer	303	204	-	217	Hoskin et al. (2006)
Autumn	-	180	-	158	Hoskin et al. (2006)
Autumn	282	-	-	191	Kusmartono et al. (1996a)
Autumn	253	-	-	175	Min et al. (1997)
Cows (l/cow/d)					
Summer	11.9	11.6	-	9.9	Minnee et al. (2012)
Autumn	10.8	-	-	10.4	Waugh et al. (1998)

2.4.2 Voluntary feed intake

Herbage DM intake (DMI) is a major determinant of animal performance by ruminants in pasture based systems (Ungar and Noy-Meir, 1988). The high FV of chicory and plantain for ruminants is largely attributed to a greater VFI in conjunction with the higher nutritive value per unit of DM eaten (Barry, 1998). Chicory and plantain swards have greater leaf mass and accessible leaves, which allow cows to take bigger bite mass than from perennial ryegrass (Gregorini et al., 2013; Stewart, 1996). Greater bite mass requires more handling to accommodate the herbage consumed by increasing chewing rate as bite mass increase (Gregorini et al., 2013; Kusmartono et al., 1996c). The chewing rate of sheep fed plantain, and deer fed chicory, is greater than perennial ryegrass (Derrick et al., 1993; Kusmartono et al., 1996b). Also, dairy cows grazing chicory or plantain made more mastications per minute leading to the ingestion of smaller particles and reducing rumination time than those grazing perennial ryegrass (Gregorini et al., 2013). Lower NDF concentration in forage is associated with less physical damage required to breakdown particle size. Consequently, the greater chewing rate of animals grazing herbs and the lower NDF content suggests animals swallow smaller particle size compared with perennial ryegrass (Gregorini et al., 2009).

Both herbs are characterised by a faster ruminal degradation and lower rumen mean retention time (Hoskin et al., 1995; Kusmartono et al., 1997) due to their high ratio of RFC:SC (Barry, 1998; Burke et al., 2000). Behavioural observations reported that red deer fed chicory, and cows fed either chicory or plantain spent less time ruminating compared with those fed perennial ryegrass (Gregorini et al., 2013; Hoskin et al., 1995; Kusmartono et al., 1997). Therefore, chicory and plantain suggest that higher intakes are possible expected to improve animal performance (Burke et al., 2000). It is essential that grazing management does not constrain VFI if a high FV is to be achieved (Kemp et al., 2010).

2.4.2.1 Diet Selection and grazing preference

Ruminants select their diet by removing some sward components, either plant parts or whole plants, in preference to others on offer (Hodgson, 1979; Parsons et al., 1991). Preference refers to what animals want to eat when no constrains alter their freedom of choice (Hodgson, 1979). A preference trial showed lambs similarly preferred chicory and plantain during spring and summer, but their preference was lower than for red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) (Pain et al., 2010). Under grazing, the preference of animals is modified by the opportunity of selection, where the sward characteristics may restrict the access to some components (Hodgson, 1985). Vegetation characteristics of the sward, such as its accessibility (vertical distribution) and availability (horizontal availability) of the sward components affect diet selection of grazing animals (Dumont, 1997; Edwards et al., 1996; Hodgson et al., 1997; Parsons et al., 1994; Ungar and Noy-Meir, 1988).

Both herbs are reported as being palatable for ruminants (Barry, 1998; Stewart, 1996). Plant palatability influences the acceptance of a species in preference to others on offer (Arnold, 1964; Heady, 1964; Hodgson, 1979). However, palatability problems with plantain have been reported during late summer and autumn (Fraser and Rowarth, 1996), and are associated with the stem development of the plant (Swainson and Hoskin, 2006). The palatability of a plant decreases as it matures, with increased lignification making the plant less acceptable by animals (Baumont et al., 2000; Gregorini et al., 2009). Animals avoid eating the stem of plants and prefer vegetative swards due stem content reduce the OMD of the herbage and hence the VFI (Waghorn and Clark, 2004).

2.5 PASTURE MANAGEMENT

2.5.1 Stem development

Stem development in chicory and plantain occurs in late spring and summer (Hare et al., 1987; Lee et al., 2015; Sanderson et al., 2003). Chicory when sown in spring will not develop stem until the following spring as this is triggered by vernalisation during winter (Moloney and Milne, 1993; Rumball, 1986). When chicory is sown in autumn, however, it will develop immature stem in the first spring (Li et al., 1994). Chicory produces a hollow stem that may achieve a height of 60 cm, but can grow to over 2 m in height if the reproductive growth is not controlled (Hare et al., 1987). During spring, chicory can develop up to 80% stem in the total yield when not grazed (Matthews et al., 1990) or up to 65% of the total herbage production when defoliating at eight week intervals (Clark et al., 1990).

Plantain has the propensity to rapidly develop stem from spring (Sanderson et al., 2003). Plantain produces more stem than chicory with greater stem growth during late spring and summer, ranging from 10 - 50% of the total yield, depending on grazing management (Lee et al., 2015). Fraser and Rowarth (1996) reported that plantain cultivar ‘Grassland Lancelot’ had a stem contribution of 60% in the sward during summer and autumn. However, Tonic Plantain was reported to have lower stem production than Grassland Lancelot plantain (Sanderson et al., 2003). The higher content of fibre and lignin and lower OMD of stem compared to leaf (Clark et al., 1990; Fraser and Rowarth, 1996; Lee et al., 2015; Li et al., 1994; Swainson and Hoskin, 2006) negatively influenced the VFI and overall FV of the forage (Hoskin et al., 2006). Stem control of chicory and plantain is important to maintain high FV (as discussed in section 2.4.2).

2.5.2 Pasture persistence

Chicory and plantain are perennial species, having a potential stand life of up to seven years (Moloney and Milne, 1993). The persistence of chicory under grazing conditions has ranged from two to three years (Charlton and Stewart, 1999; Li and Kemp, 2005), while plantain has shown to persist for at least three years (Kemp et al., 2014). Pasture persistence is affected by many factors, such as establishment failure, grazing management, fertilizer application, diseases, weather conditions as well as animal diet selection (Li and Kemp, 2005). Lack of persistence occurs when the sown species is replaced by weeds due to a decline in the plant density of the sown species leading to significant herbage production loss (Parsons et al., 1991; Tozer et al., 2011). Therefore, it is necessary to maintain adequate plant numbers in the sward due to the close relationship between plant density and herbage production (Li et al, 1997a). Both chicory and plantain plant density declines over time from the establishment year, independent of grazing frequency or grazing intensity (Glassey et al., 2013; Labreveux et al., 2004; Li et al., 1997b; Sanderson et al., 2003).

Chicory loses approximately 35% of its initial plant density during its first growing season with the largest decline in late spring and summer (Li et al., 1997b). During the first two growing seasons, chicory swards have higher plant density (plants/m^2) and maintain their herbage production compared with chicory swards in their fourth growing season which accumulated only half of its herbage production (Li et al., 1997a). Similarly, Hume et al. (1995) reported that chicory dominated the swards in mixed pastures during the first three growing seasons, but its herbage production was reduced by the fourth growing season. Once chicory plant density in the sward declines to 25 plants/m^2, its herbage production is likely to be less than half of its yield potential (Li and Kemp, 2005).

Previous research in New Zealand evaluating chicory and plantain has reported higher plantain plant density and has better persistence than chicory (Glassey et al., 2013; Lee et al., 2015; Powell et al., 2007). Plantain has a 14% plant loss during the first growing season and hence is considered to persist better than chicory under sheep grazing (Powell et al., 2007). Moreover, seedling recruitment of plantain enables this herb to reseed in pasture as a mechanism to maintain its plant density (Ayala et al., 2011b; Derrick et al., 1993), which is unlikely for mature chicory (Li and Kemp, 2005). The persistence of chicory appears to depend more on the survival of the original plants (Li and Kemp, 2005).

2.6 GRAZING MANAGEMENT

Grazing management for chicory and plantain aims to maximize leaf growth and minimize stem development (Clark et al., 1990; Hume et al., 1995; Lee et al., 2015; Li and Kemp, 2005). Grazing recommendations for frequency and intensity are of importance to maximise the pasture persistence.

Grazing or defoliation frequency has a direct effect on herbage production. In New Zealand, Clark et al. (1990) showed that chicory yielded 2.2, 3.2, 4.8, and 6.4 t DM/ha at defoliation intervals of one, two, four, and eight weeks, respectively during late spring. Moreover, from late spring to autumn, chicory had greater accumulated herbage mass under four week intervals (9.6 t DM/ha) compared to one or two week intervals yielding 4.9 and 6.4 t DM/ha, respectively (Li et al., 1997b). Similarly, in the USA, chicory accumulated a greater herbage mass with defoliation intervals of four to five weeks (7.8 and 7.9 t DM/ha, respectively) compared to two week intervals (6.6 t DM/ha) over two growing seasons (Volesky, 1996). Herbage production with five week intervals have shown that chicory yielded 26% more (Belesky et al., 1999) and up to 50% more compared to three week intervals (Labreveux et al., 2004; Sanderson et al., 2003). When chicory was defoliated at different extended leaf heights (ELH), its herbage production was greater for defoliation at 35 or 55 cm (11.5 and 12.5 t DM/ha, respectively) compared to 15 or 25 cm (10.6 t DM/ha) (Lee et al., 2015).

Plantain yielded more when defoliated every five weeks compared to more frequent defoliation intervals (every three weeks) (Sanderson et al., 2003; Labreveux et al., 2004). In contrast, Ayala et al. (2011a) obtained a greater plantain herbage production (10.6 t DM/ha) with frequent grazing (three week intervals) compared to infrequent grazing (six week intervals; 5.6 t DM/ha).

Short rest periods have a direct effect in reducing the herbage production in comparison to long rest periods. However, leaf yield rather than total yield (leaf + stem) is of importance to maintain good quality forage (Li and Kemp, 2005), and a reduction in the rest period limits partitioning of DM into reproductive structures (Labreveux et al., 2004). Greater grazing intervals increased stem development and also increased total herbage production in pasture (Lee et al., 2015; Li and Kemp, 2005; Clark et al., 1990). In relation to Puna chicory, Clark et al. (1990) reported 60% leaf in the total DM with four week intervals, but decreasing to 36% with eight week intervals of defoliation, and suggested that maximum leaf yield occurred after five weeks of regrowth to obtain the desirable ratio leaf and stem (70:30). However, a four week defoliation interval had the highest leaf and stem mass and a high ratio of leaf to stem (85:25) (Li et al., 1997b). Recently, Lee et al. (2015) observed that in a sward of spring sown of Choice chicory, the frequency of defoliation did not affect stem development, which yielded only 5% of total DM during the first growing season; but in the following year, stem production increased from 12 to 31% of the total annual yield for increasing defoliation levels of 15 to 45 cm ELH.

In plantain, the yield of stem increases with increasing defoliation intervals from its first growing season. During its second growing season, stem as a percentage of the total annual yield was 10, 26, 42, and 48% for plantain defoliated at 15, 25, 35, and 45 cm of ELH, respectively (Lee et al., 2015). Reproductive stems increased in summer as a consequence of frequent defoliation intervals (1584 and 924 kg DM/ha of stem for three and six week intervals, respectively) and less intense defoliation heights (899 kg DM/ha for 20 mm and 1461 kg DM/ha for 100 mm residual). The greatest leaf yield over two years was obtained from plantain swards defoliated at 35 and 45 cm ELH, the same treatments that maximised stem growth (Lee et al., 2015).

Grazing intensity in chicory does not appear to affect its herbage production (Li et al., 1997a; Lee et al., 2015); however, the proportion of stem increased as grazing intensity decreased (Clark et al., 1990; Li et al., 1994). Grazing chicory swards very hard (0 - 50 mm residual height) or hard (50 - 100 mm residual height) produced the lowest stem, whereas a lax - grazing (150 - 200 mm residual height) had the greatest stem production. Leaf mass production of chicory when cutting to ground level was similar for defoliation intervals of one, two, and four weeks (70% leaf) but when cut to a residual height of 100 mm the stem contribution was lower at one and two week intervals (26% and 34%, respectively) compared to four and eight week intervals (60% and 63%, respectively) (Clark et al., 1990). Moreover, a hard grazing (50 mm residual height) in summer had greater leaf and lower stem production than grazing to 80 mm residual height (Lee et al., 2015). Tozer et al. (2012) reported a similar herbage mass for chicory under lax (100 mm residual height) or intense (50 mm residual height) grazing in early summer (January), but in contrast, afterwards (February and March), chicory herbage mass was higher under lax compared with intense grazing. In the case of chicory, taller stubble can lead to a non-desirable canopy structure, with regrowth occurring from buds left on the flower stalk instead of coming from the crown (Li et al., 1997b). If optimal grazing control does not occur in late spring or summer, mechanical topping is recommended to remove stem (Li and Kemp, 2005).

Chicory can be grazed to ground level at each grazing as it regenerates new shoots from the basal crown (Rumball, 1986). However, excessively frequent cutting or grazing is detrimental to the persistence of chicory. The combination of a three week grazing frequency at a 50 mm residual height has been shown to be detrimental to chicory (Labreveux et al., 2004). Severely grazed plants are been forced to mobilize carbohydrate reserves for regrowth, and a three week defoliation interval does not allow sufficient time for the plant to achieve maximum DM accumulation.

Plantain grazed at an intensity of 50 mm residual height increased total DM yield compared to the residual height of 80 mm (Lee et al., 2015). Labreveux et al. (2004) did not observe any effect of residual height (50, 100, or 150 mm) on plantain yield during spring and summer. Ayala et al. (2011a) also reported that grazing plantain either hard (30 mm residual) or lax (100 mm residual) did not affect DM production in spring and summer. Conversely, Lee et al. (2015) reported that in spring a residual height of 50 or 80 mm had similar DM production, but in summer (4.8 t DM/ha) and autumn (1.5 t DM/ha), a lower residual height (50 mm) increased the DM production compared to those defoliated to residual height of 80 mm (4.3 t DM/ha in summer and 1.1 t DM/ha in autumn). During summer, infrequent (six week intervals) and lax (100 mm residual) grazing intensity reduced herbage DM production; in autumn, frequent (three week intervals) and hard (20 mm residual) grazing increased herbage DM production by 141% (Ayala et al., 2011a). Clearly, the impact of grazing intensity on plantain yield is variable, and grazing management requires further investigation and refinement (Lee et al., 2015; Kemp et al., 2014). A post grazing height of 50 mm limits lambs intake, with herbage masses of 1200 to 1400 kg DM/ha and a residual height of 85 mm maximizing lamb intake (Moorehead et al., 2002). Plantain, as a monoculture, improves lamb performance when grazed lightly (80 - 100 mm residual height), but the proportion of old and ungrazed leaves also increases, resulting in poor utilisation of the pasture (Kemp et al., 2013). This has practical disadvantages, such as poor utilization and poor stem control making the forage too mature for high intakes.

2.7 HERB-CLOVER MIX PASTURES

To develop more productive and sustainable pasture systems, the use multi-species pastures, with four or more species, combining grasses, legumes and herbs species, emerged as an alternative with improved DM production in comparison to perennial ryegrass/white clover pasture (Sanderson et al., 2005). Increasing plant diversity was positively related to higher DM production, greater pasture stability, and reduced weed invasion (Daly et al., 1996; Deak et al., 2007; Ruz-Jerez and Ball, 1991; Sanderson et al., 2005).

In New Zealand, Kemp et al., (2010) suggested that these herbs species used in a herb-clover mix, sowing chicory, plantain, white clover, and red clover, could provide a greater production of high quality herbage and increase the length of the growing season. Herb-clover mix pastures from spring to autumn can produce as much dry matter, or more, than the annual yield of perennial ryegrass (Goh and Bruce, 2005). Research on the FV of herb-clover mix pastures has shown to be greater in sheep production systems when compared to perennial ryegrass (Golding et al., 2011; Hutton et al., 2011; Kemp et al., 2010; Kenyon et al., 2010). Additionally, research on mixed pastures has been shown to reduce the excretion of N in the urine of dairy cows (Totty et al., 2013; Woodward et al., 2013). These suggest that herb-clover mix pastures have the potential to improve milk production with environmental advantages by reducing the N excreted in the urine to the environment in dairy systems.

2.8 CONCLUSIONS AND RESEARCH OBJECTIVES

Chicory and plantain are able to grow high quality herbage during summer/autumn. Their herbage production, nutritive value, and animal production have been documented in the literature primarily in relation to sheep and deer. Both herbs combined with red and white clover, in an herb-clover mix pasture, may provide a more productive pasture, of higher nutritive value, than pure chicory or plantain. Chicory is reported as being less persistent than plantain and has poor persistence when grazed by dairy cows; while for plantain and herb-clover mix pastures, there is limited information on the effects of grazing management and persistence in dairy systems. The presence of secondary compounds in plantain, with antimicrobial activity, may have implications for rumen fermentation and function. The focus and objectives for the experimental chapters in this thesis are as follows:

I. To evaluate the effect of grazing frequency on the production, persistence, plant and root development of chicory and plantain, as pure swards and in an herb-clover mix pasture with dairy cows throughout two growing seasons. (Chapter 3).

II. To compare the nutritive value of chicory, plantain, and herb-clover mix pastures (Chapter 4).

III. To examine the diet selection and grazing preference of dairy cows for chicory and plantain, as pure swards and in an herb-clover mix pasture (Chapter 5).

IV. To determine the bioactive compounds present in plantain, and investigate their effect of on rumen *in vitro* fermentation (Chapter 6).

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3 EFFECT OF GRAZING FREQUENCY BY DAIRY COWS ON HERB BASED PASTURES THROUGHOUT TWO GROWING SEASONS

3.1 ABSTRACT

Both chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) are used by dairy farmers to supplement lactating cows over summer and autumn periods, but there have been many cases of poor chicory persistence with current management practices and poor understanding of plantain grazing management. Additionally, there is an increased interest in the use of mixed swards. The objective of this experiment was to evaluate the herbage production and persistence of three pasture treatments: (i) chicory; (ii) plantain; and (iii) an herb-clover mix, containing chicory, plantain, red clover (*Trifolium pratense* L.), and white clover (*T. repens* L.) when managed with dairy cows at two grazing frequencies (two or four week intervals) throughout two growing seasons (2011-2012 and 2012-2013). Perennial ryegrass (*Lolium perenne* L.)/white clover pasture was also included in order to establish a benchmark, but best practice management was applied to this pasture rather than grazing frequency. All pastures were established in October 2011. The pasture treatments were arranged in a randomised block design (n=5) with factorial arrangement (3 pastures x 2 frequencies) in a plot of 11.5 m x 26 m. The pasture treatments were grazed every two and every four weeks to a residual height of between 70 - 100 mm. Grazing frequency affected the persistence of chicory but not plantain. Grazing chicory every two weeks resulted in plants having smaller taproots and storing less carbohydrate reserves than those grazed every four weeks. Chicory, especially when grazed every two weeks, failed to persist into the second growing season. Plantain was more flexible with grazing frequency, maintaining its plant density and taproot development irrespective of grazing frequency. Overall, plantain and the herb-clover mix persisted better than chicory and were more productive than chicory and perennial ryegrass/white clover pasture in both growing seasons.

Keywords: grazing frequency, dairy cow, chicory, plantain, herb-clover mix

3.2 INTRODUCTION

Feed deficit and lower animal production with perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) pasture increases from summer to autumn due to soil moisture deficit (Kemp et al., 2010; Labreveux et al., 2004; Li and Kemp, 2005; Sanderson et al., 2003). As result, chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) are being widely used by dairy farmers to supplement dairy cows during this period (Glassey et al., 2013; Lee et al., 2015). These species have deeper rooting systems and are more droughts tolerant than perennial ryegrass/white clover (Lee et al., 2015). Chicory and plantain produce greater feed quality supply and improve dairy cows performance during summer and autumn (Minnee et al., 2012; Waugh et al., 1998). Despite these benefits, pasture persistence and, therefore, herbage production is affected by grazing management practices. Grazing management recommendations have been elucidated for chicory (Clark et al., 1990; Li et al., 1994; 1997b; c; Powell et al., 2007), but little is known about plantain grazing management.

Both chicory and plantain are perennial herbs. The annual dry matter (DM) production obtained in New Zealand has ranged from 9 - 14 t DM/ha for chicory (Li and Kemp, 2005; Powell et al., 2007) and up to 17 t DM/ha for plantain (Powell et al., 2007) with their greatest herbage being accumulated during late spring and summer (Lee et al., 2015; Li and Kemp, 2005; Powell et al., 2007). These herb species when sown with red clover (*T. pratense* L.) and white clover in a herb-clover mix pasture may improve herbage production of high quality throughout the growing season (Kemp et al., 2010). However, there is limited information of herb-clover mix pastures on annual DM production or being more productive compared to pure swards of chicory or plantain for dairy systems.

Fully productive pastures of chicory and plantain have shown to persist from two to five years (Charlton and Stewart, 1999; Kemp et al., 2014; Li and Kemp, 2005). To ensure high yields pastures it is necessary to maintain adequate plant density in the sward, which has been stated as above 50 plants/m^2 for chicory (Li and Kemp, 2005), but not yet been established for plantain. Chicory has been reported to persist for three years under sheep and deer grazing (Li and Kemp, 2005). However, under grazing with dairy cows, chicory has been described to persist one to two years (Rollo et al., 1998; Glassey et al., 2012). Compared to chicory, plantain has shown to have greater plant density and DM production (Glassey et al., 2013; Lee et al., 2015; Powell et al., 2007). Grazing plantain with sheep during one year persisted better than chicory (Powell et al., 2007). Farmers have also been reported to believe that plantain is more persistent than chicory (Tozer et al., 2011). Evidence from trials with dairy cows has shown a higher decline in the plant density of chicory compared to plantain during the first year (Glassey et al., 2013), which suggests that plantain may persist better than chicory under grazing with dairy cows.

Chicory and plantain are taproot species that utilise their root carbohydrate reserves for regrowth after grazing, so need time to recover these root reserves (Kemp et al., 2010). Best grazing management practices for chicory recommends grazing every four to five weeks, but not grazing bellow 50 mm of residual height (Li and Kemp, 2005). A frequent grazing is detrimental for the persistence of chicory due to the depletion of their root reserves (Li et al., 1997a). In relation to plantain there is still limited information on the effect of grazing management on its persistence, taproot development and carbohydrate reserves. The objective of this study was to evaluate the effect of grazing frequency (every two and four weeks) on the DM production, pasture persistence, taproot development and carbohydrate reserves of chicory and plantain, as pure swards and in a herb-clover mix pasture rotationally graze with dairy cows throughout two growing seasons.

3.3 MATERIALS AND METHODS

3.3.1 Experimental site and treatments

A grazing experiment was conducted during 2011-2012 (first growing season) and 2012-2013 (second growing season), at No1 Dairy Unit at Massey University in Palmerston North, New Zealand (40° 22′ S, 175° 36′ E). Three pasture treatments were evaluated: (i) chicory, (ii) plantain, and (iii) an herb-clover mix pasture, containing chicory, plantain, red clover and white clover, under two grazing frequencies; every two or four weeks. Perennial ryegrass/white clover pasture was also established and grazed to provide a benchmark (Table 3-1).

Table 3-1 The pasture treatments and perennial ryegrass/white clover pasture, cultivars and sowing rates.

Pasture	Species and cultivar	Sowing rate kg seed/ha
Chicory	Chicory cv. ‘Grassland Choice’	6
Plantain	Plantain cv. ‘Ceres Tonic’	8.4
Herb-clover mix	Chicory	6
	Plantain	6
	Red clover cv. ‘Sensation’	6
	White clover cv. ‘Emerald’ and cv. ‘Bounty’	4
Perennial ryegrass/ white clover	Ryegrass cv. ‘Alto’ (AR37),	25
	White clover cv. ‘Emerald’ and cv. ‘Bounty’	4

3.3.2 Experimental design

The pasture treatments were established in a randomised block (n=5) design with a factorial arrangement (two grazing frequencies) of the pastures (30 plots in total), plus five plots of perennial ryegrass/white clover sown on a side of the pasture treatments in order to have a benchmark (Figure 3-1). The pastures were sown in plots with an area of 11.5 by 26 m (≈300 m^2). The perennial ryegrass/white clover plots did not have a factorial arrangement and was grazed according to its best practice management (herbage mass of 2500 - 3000 kg DM/ha) rather than grazing frequency.

3.3.3 Site preparation and sowing

The pastures were sown in spring (10 October, 2011). A month before the sowing, the experimental site was sprayed with glyphosate (3 L/ha) to eliminate the existing vegetation. The soil type is a Manawatu silt loam over sand. Soils tests to 75 mm depth were conducted at the experimental site and showed levels of soil fertility of Olsen phosphate of 31 ppm, sulphate of 13 ppm, potassium of 0.42 me/100g, and pH 5.7. Before sowing, the paddock was ploughed, power harrowed, fertilised with 27, 18, 18 kg/ha nitrogen (N), phosphorus (P) and potassium (K), respectively, and rolled to form a fine, even seed bed. The seed was sown on the soil surface then rolled directly after sowing with a 15-row cone seeder. Germination test and a thousand seed weights of chicory and plantain seed were carried out to establish sowing rates (Table 3-1). The germination rate (%) of chicory and plantain was determined from 100 seed (n=5) maintained at 30 °C during the day and 20 °C during the night. The seed of chicory germinated after 3, 5, 7, and 14 days were counted. For plantain, the counting of seed germinated was after 4, 7, 14, and 21 days. Sowing rates (Table 3-1) were, therefore, based on measured thousand seed weights of chicory (1.49 g) and plantain (2.12 g) for the pure swards and the herb-clover mix pastures to ensure a similar plant density for each of the herb species at a determined 83% and 90 % germination rate for chicory and plantain, respectively.

3.3.4 Grazing management

The grazing of the herbs and herb-clover mix pastures was strictly every two and every four weeks. Plots were grazed with lactating dairy cows immediately after the morning milking until the swards achieved a residual height of 70 - 100 mm (5 - 6 h). The first grazing event, in each growing season, was done with the same number of cows, in both the two and four week frequency plots. From the second grazing onwards, the cow number used at each two and four week grazing event was adjusted according to the combination of herbage mass available and the herd size of the dairy farm at the time of grazing.

3.3.4.1 2011-2012 growing season

The grazing of the herbs and herb-clover mix pastures in first growing season (2011-2012) started on 20 December, 2011. The two week frequency plots of chicory and the herb-clover mix were grazed until 12 April, 2012 (nine grazing) and for plantain until 26 April, 2012 (ten grazing). The two week frequency plots finished before it had been scheduled because of the slow growth of these pastures. The four week frequency plots were grazed until 6 June, 2012. Plantain and the herb-clover mix were grazed on seven occasions and only six times for chicory (not grazed on 9 May, 2012). During the first growing season the perennial ryegrass/white clover plots were grazed on six occasions (6 December, 2011 until 6 June, 2012). Over the following months (June-July, 2012), the pastures were not grazed.

3.3.4.2 2012-2013 growing season

The second growing season (2012-2013) started on 22 August, 2012, for plantain and the herb-clover mix pastures, and one month later, on 19 September, 2012, for chicory pastures. The second growing season was completed on 30 May, 2013. Plantain and the herb-clover mix pastures were grazed 20 and 11 times for the two and four week frequency, respectively. Chicory was grazed 18 and 10 times for the two and four week grazing frequency, respectively. While, perennial ryegrass/white clover pasture were grazed on 13 occasions during this growing season. In March, 2013, the grazing schedule protocol was disrupted, with the grazing of the two week frequency plots cancelled; this was due to the slow growth of the pastures as a result of a six week drought over the summer. Although the experimental site was irrigated, it was not possible to apply sufficient water in the time available for irrigation on the two week frequency treatments at the height of the drought.

3.3.5 Pasture management

3.3.5.1 2011-2012 growing season

Despite the spring sowing (October, 2011), many of the chicory plants were vernalised due to the unusually cold spring and as a consequence, there were many flowering stems produced in January, 2012. It was necessary to mow (15 February, 2012) the four week frequency chicory plots but not the two week frequency plots and the herb-clover mix in both the two and four week grazing frequency plots. The perennial ryegrass/white clover plots were mowed on two occasions, 15 February and 20 April, to control the high infestation of broad leaved dock (*Rumex obtusifolius* L.).

3.3.5.2 2012-2013 growing season

Before starting the second growing season (16 August 2012), 50 kg N/ha as urea was applied to all the pastures. Chicory plants exhibited signs of a bacterial disease (*Pseudomona cichorii*) before starting the second growing season; hence on 28 August, 2012, all chicory plots were sprayed with 6 kg/ha of Oxi-Cup® WG (500 g/kg of copper as copper oxychloride). To control the growth of grasses, the pasture treatments were treated with Gallant Ultra (0.5 L/ha) and Uptake spraying oil (200 mL/ha). Flowering chicory stems were controlled by mowing the chicory and the herb-clover mix plots, in both the two and four week grazing frequency, immediately after grazing on 13 December, 2012.

The experimental site was irrigated in January, 2012 (25 mm) and again in March, 2012 (50 mm). Despite irrigation, it was necessary to cancel one grazing (21 March, 2013) of all plots in the two week grazing frequency due to drought conditions reducing herbage growth.

3.3.6 Sward measurements

Herbage mass was determined pre-grazing and post-grazing by cutting to ground level two samples randomly chosen per plot (0.1 m^2 quadrats) using an electric shearing handpiece. The pre-grazing samples were taken the day before the grazing and the post-grazing samples were cut immediately after grazing. The samples were washed to remove soil contamination and DM was determined after oven drying at 70 °C for approximately 48 h. Herbage mass (kg DM/ha) accumulated between grazing was calculated as the pre-grazing herbage mass minus the post-grazing herbage mass of the previous grazing. The net DM accumulation during both growing seasons was calculated as the herbage mass accumulated between each grazing, that was positive, summed over time (Hodgson, 1979).

The botanical composition of the pasture treatments was evaluated throughout the first growing seasons in December, 2011; March, 2012 and May, 2012. During the second growing season every two months for plantain and the herb-clover mix (August, 2012; November, 2012; February, 2012; May, 2013), and monthly for chicory. Botanical samples were taken by cutting a strip (50 x 10 cm) beside each herbage mass pre-grazing quadrat. The botanical samples were manually separated into the following categories: herbs (chicory and plantain); clovers (white and red clover); weeds (grass and broadleaf weeds); and dead material and oven-dried individually at 70 °C (approximately 48 h). Then, the proportion for each category in the DM was calculated.

Plant density (plants/m^2), shoot density (shoots/m^2), and plant size (shoots/plant) of chicory and plantain in the pure swards and in the herb-clover mix was evaluated at the beginning (December, 2011) and at the end (May, 2012) of the first growing season. During the second growing season, samples were taken every two months (August, 2012; November, 2012; February, 2012; May, 2013) for plantain and the herb-clover mix, and monthly for chicory. Two randomly selected square areas (0.04 m^2) in each plot were dug out with a spade, and the number of chicory or plantain plants was counted, recording for each plant the number of primary and secondary shoots. The taproot diameter (mm) of chicory and plantain was also measured (at the top of the taproot) at these times.

During the second growing season fresh root samples of chicory and plantain were taken for carbohydrate reserves analysis in August, 2012; November, 2012; February, 2012; May, 2013. Two randomly selected areas (0.04 m^2) were dug out with a spade from in each plot and bulked together. Harvests were consistently performed during the morning to limit diurnal variation in WSC concentration of plants (Fulkerson and Slack, 1994). The top 5 cm of root of chicory and plantain were frozen at -20 °C immediately after being washed, then freeze-dried and ground to pass through a 1 mm sieve for analyses. Root samples of chicory and plantain were analysed using an enzymatic method for glucose, fructose and sucrose concentrations (Sekin, 1978) and for fructan content (McCleary and Blakeney, 1999).

Total rainfall (mm), maximum and minimum air temperature (°C), and soil temperature (°C) data from October, 2011 to May, 2013 were obtained from a weather station, located at 400 m from the experimental site.

3.3.7 Statistical analysis

All data were analysed using PROC MIXED for repeated measures in SAS (SAS Institute, 2009). The net herbage accumulation of the pasture treatments was analysed using a model that included the fixed effects of pasture treatments (chicory, plantain, and herb-clover mix), grazing frequency (two and four weeks) and growing season (2011-2012 and 2012-2013) and their interactions. Growth rate, herbage mass pre-grazing and the botanical composition of the pasture treatments was analysed using a model that included the fixed effects of pasture treatment, grazing frequency and month. Plant density, plant size, taproot diameter, and carbohydrates of reserves of chicory and plantain were evaluated in a model that included the fixed effects of pasture type (pure sward and herb-clover mix), grazing frequency, month and their interactions. Perennial ryegrass/white clover was not included in the statistical analysis due to its grazing management not being every two or four weeks.

3.4 RESULTS

3.4.1 Climatic conditions

The total monthly rainfall, the mean monthly air and soil temperatures during the course of the experiment and the 10-year mean (1999-2009) as the long term averages (LTA) are presented in Table 3-2. The mean monthly air and soil temperatures throughout the experiment were similar to the LTA, whilst rainfall was the most variable of the weather characteristics in both growing seasons.

During the first growing season (2011-2012) the total rainfall was 674 mm (October, 2011 to May, 2012), which was 11% greater than the LTA (610 mm) due to a wet summer (January to March, 2012). The total rainfall during summer was 27% greater than the LTA (170 mm), and irrigation was not necessary as the only water deficit of consequence was in April to May, 2012 when the soil temperature was cooling down. During the second growing season (2012-2013), the total rainfall (August, 2012 to May, 2013) was lower (632.6 mm) than the LTA (786.2 mm), due to spring and summer being particularly dry. Spring precipitation (August to December, 2012) was only 59% of the LTA (370 mm), and the experimental site was irrigated in January, 2013 (25 mm). A continued lack of rainfall in summer (summer precipitation was only 66% of the LTA) caused the North Island to officially declare drought conditions and it was, consequently, necessary to irrigate again in March, 2013 (50 mm) and to cancel one grazing event in the two week grazing frequency treatment.

Table 3-2 Total monthly rainfall (mm), mean monthly maximum and minimum air temperature (°C), mean monthly soil temperature (°C) between October, 2011 and May, 2013 and long term average (LTA).

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
						Rainfall						
2011										145.6	134.2	87.2
2012	87.2	72.0	57.0	28.2	62.2			98.2	58.8	64.6	20.2	100.2
2013	29.0	34.0	37.2	128.2	62.2							
LTA[1]	58.1	64.9	46.7	65.9	85.7	107.4	94.0	94.6	82.3	110.3	79.4	98.4
						Maximum air temperature						
2011										17.3	17.9	21.7
2012	21.1	21.1	19.7	19.5	15.0			14.4	15.2	16.1	17.6	22.3
2013	23.3	24.1	23.6	19.6	14.9							
LTA[1]	22.5	23.2	22.1	18.7	16.3	13.7	13.1	13.7	15.5	16.6	18.1	20.6
						Minimum air temperature						
2011										8.4	10.2	11.9
2012	12.1	12.2	10.8	8.4	3.8			5.6	5.7	7.1	7.8	12.5
2013	11.7	12.2	11.1	10.8	3.8							
LTA[1]	12.5	13.0	11.7	8.4	7.0	4.6	4.3	4.4	6.2	7.7	9.1	11.7
						Average soil temperature						
2011										12.9	14.5	18.3
2012	18.0	17.2	15.3	13.7	10.3			8.9	9.9	12.0	14.5	18.4
2013	18.3	19.3	17.7	14.3	9.2							
LTA[1]	18.4	18.5	16.5	12.8	10.6	8.1	7.2	7.9	10.2	12.0	14.2	16.8

[1] LTA: long term average, 10 years mean (1999-2009).

3.4.2 Herbage production and growth rate

The net herbage accumulated was different (P<0.05) between the pasture treatments in both growing seasons (Table 3-3). Plantain and the herb-clover mix pastures were more productive (P<0.05) than chicory in the first (2011-2012) and second (2012-2013) growing seasons. Perennial ryegrass/white clover had a herbage production numerically greater than chicory, but lower than plantain and the herb-clover mix pastures. During the second growing (2012-2013) season the net herbage accumulated for the pasture treatments was greater (P<0.05) and numerically greater for perennial ryegrass/white clover compared to the first (2012-2013) growing season. There was no effect (P>0.05) on the net herbage accumulated by grazing frequency or by the interaction between pasture treatments and grazing frequency in either growing seasons.

Table 3-3 Net herbage mass accumulated (kg DM/ha) in the pasture treatments and perennial ryegrass/white clover pasture and when grazing the pasture treatments every two and every four week during the first (2011-2012) and second (2012-2013) growing seasons (mean ± SEM[1]).

	Growing season	
Treatment	2011-2012	2012-2013
Pastures		
Chicory	9825 ± 356 [b]	15016 ± 586 [b]
Plantain	13166 ± 614 [a]	18623 ± 744 [a]
Herb-clover mix	13016 ± 433 [a]	17751 ± 902 [a]
Perennial ryegrass/white clover [2]	11898 ± 580	16465 ± 967
Grazing frequency		
2 weeks	11112 ± 707	17066 ± 628
4 weeks	11893 ± 671	17193 ± 864

[a, b] superscripts indicate values within column that are significantly (P<0.05) different

[1] SEM: standard error of the mean.

[2] Perennial ryegrass/white clover was not included in the statistical analysis.

The growth rate (kg DM/ha/day) was greater (P<0.05) for plantain and the herb-clover mix pastures than chicory regardless of grazing frequency (Figure 3-1). There were no treatment interaction for the growth rate of the pasture treatments.

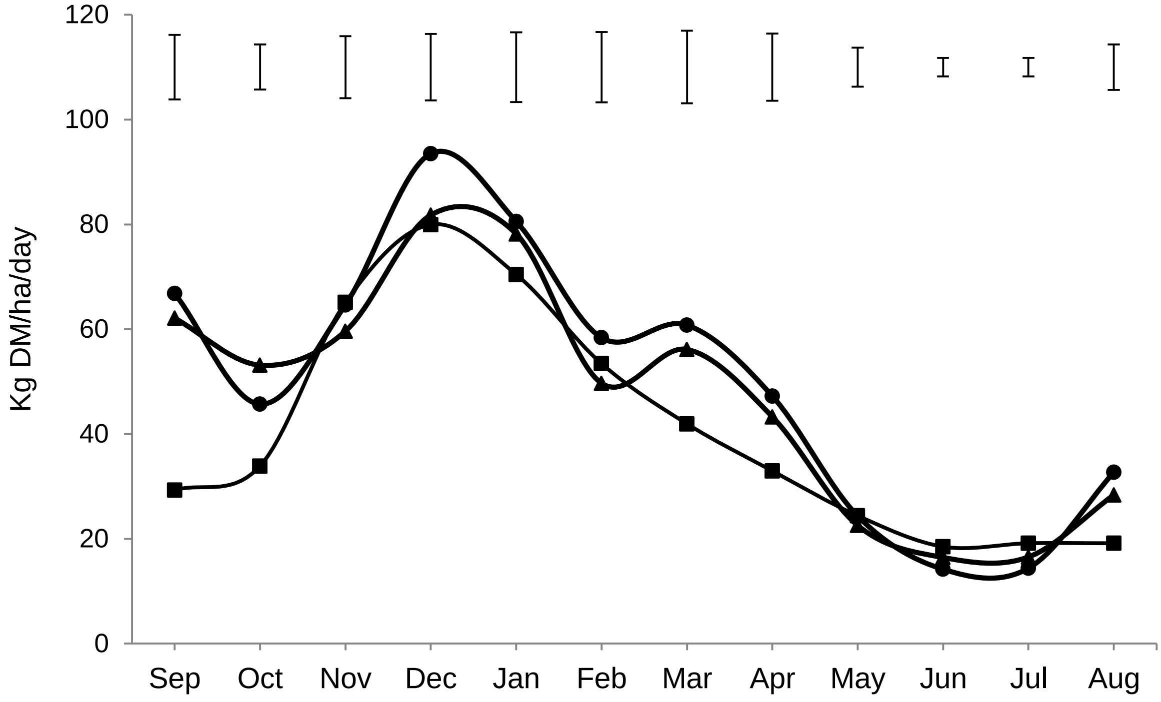

Figure 3-1 Monthly growth rate (kg DM/ha/day) in chicory (■), plantain (●), and herb-clover mix (▲) pasture. Vertical bars represent the standard error of the mean (SEM).

When the pre-grazing herbage mass of the pasture treatments was compared between grazing frequencies throughout the first (2011-2012) and second (2012-2013) growing seasons (Figure 3-2A), the herbage mass pre-grazing was greater ($P>0.05$) in the pasture treatments grazed every four weeks compared to those grazed every two weeks from January, 2012 to March, 2012 of the first (2011-2012) and from October, 2012 to May, 2013 of the second growing season (2012-2013).

The mean herbage mass pre-grazing of the pasture treatments was affected by a two-way interaction ($P<0.05$) between grazing frequency and pasture treatment (Figure 3-2B). During the first growing season (2011-2012), the herbage mass pre-grazing of plantain and the herb-clover mix pastures was greater ($P<0.05$) than for chicory in the four and two week grazing frequency treatments; however, the herbage mass pre-grazing of chicory grazed every four weeks was similar to plantain and the herb-clover mix grazed every two weeks (Figure 3-2B, 2011-2012 growing season). During the second growing season (2012-2013), the herbage mass pre-grazing of plantain and the herb-clover mix pastures was greater ($P<0.05$) than chicory grazed every four weeks, whereas the herbage mass was similar ($P>0.05$) in the three pasture treatments when grazed every two weeks but lower ($P<0.05$) than those pastures grazed every four weeks (Figure 3-2A, 2012-2013 growing season). There was no effect ($P>0.05$) on the herbage mass pre-grazing by the interaction between pasture treatments and month nor between pasture treatments, grazing frequency, and month.

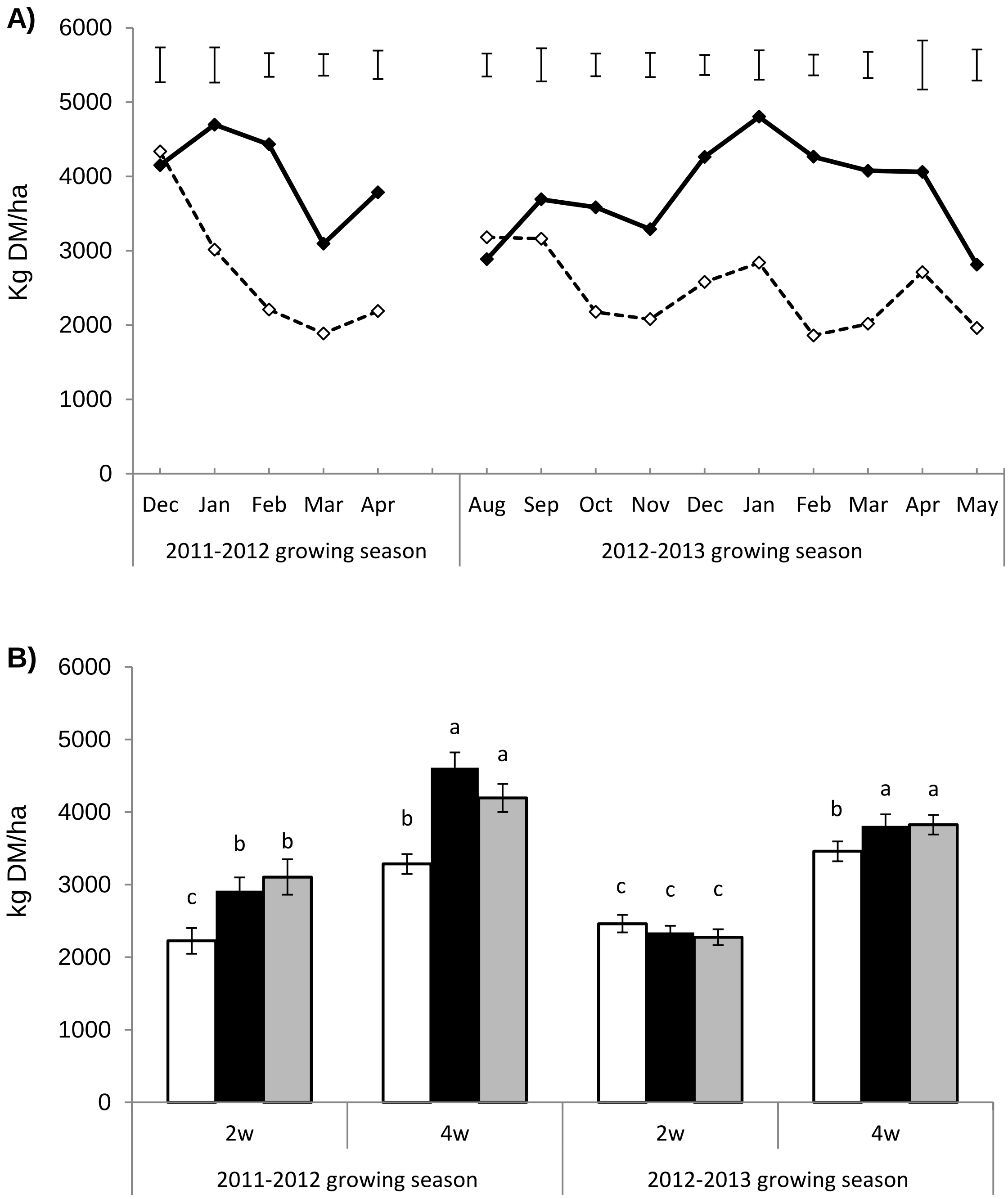

Figure 3-2 Herbage mass pre-grazing (kg DM/ha) in: A) the pasture treatments grazed every two (dashed lines) and every four weeks (solid lines) throughout the first (December, 2011 to April, 2012) and second (August, 2012 to May, 2013) growing seasons, and in B) chicory (white), plantain (black), and the herb-clover mix (grey) pastures when grazing at two (2w) or four week (4w) frequencies in the first (2011-2012) and second (2012-2013) growing seasons. Vertical bars represent the standard error of the mean (SEM). Values noted with *a, b, c* are significantly (P<0.05) different in each growing season.

3.4.2.1 Botanical composition of the pasture treatments

The botanical composition of the herbs and herb-clover mix pastures throughout the course of the experiment is presented in Figure 3-3. In the first (2011-2012) and second (2012-2013) growing seasons, all pasture treatments had an appropriate proportion of the sown species at the beginning of both growing seasons (December, 2011; August, 2012). In the chicory swards there was a lower ($P<0.05$) proportion of chicory (approximately 50%) compared to the proportion of plantain (approximately 76%) in the plantain swards in both growing seasons (Figure 3-3A and B). While in the herb-clover mix, the proportion of the sown species (chicory, plantain, red and white clover) at the beginning of both growing seasons (approximately 84%) was greater ($P<0.05$) than the proportion of chicory and plantain in the pure swards (Figure 3-3C).

Grazing frequency affected the botanical composition of the pasture treatments in both growing seasons. In the pure swards grazing every two weeks reduced ($P<0.05$) the proportion of chicory and plantain and increased ($P<0.05$) the proportion of white clover and weeds compared to those swards grazed every four weeks in both growing seasons (Figure 3-3A and B). However, in the herb-clover mix, the proportion of the herbs was unaffected ($P>0.05$) by grazing frequency in either of the growing seasons, but grazing frequency altered ($P<0.05$) the proportion of white and red clover (Figure 3-3C). The proportion of white clover was greater ($P<0.05$) in the swards grazed every two weeks compared to those grazed every four weeks, whereas, the proportion of red clover was greater ($P<0.05$) in the herb-clover mix grazed every four weeks than the two weeks grazing frequency over both growing seasons (Figure 3-3C).

During the first growing season (2011-2012), the proportion of chicory was similar (P>0.05) between months in both grazing frequencies, with chicory swards grazed every four weeks maintaining higher proportion of chicory (approximately above 50%) over all months (P<0.05) than in chicory swards grazed every two weeks (averaging 38%) (Figure 3-3A; 2011-2012). The decline in chicory caused an increase (P<0.05) in the proportion of white clover and weeds in the swards grazed every two weeks. In plantain swards, the proportion of plantain decreased (P<0.05) by 30% in the swards grazed every four weeks (from 76% to 53%) and by 55% in the swards grazed every two weeks (from 73% to 36%) throughout the first growing season (Figure 3-3B; 2011-2012).

In the herb-clover mix, during the first growing season (2011-2012), the proportion of chicory decreased (P<0.05), but remained similar (P>0.05) throughout the second growing season (Figure 3-3C); whereas the proportion of plantain in the herb-clover mix increased (P<0.05) during the first growing season (2011-2012) and decreased (P<0.05) during the second growing season (2012-2013) (Figure 3-3C).

The contribution of dead material in the swards was not affected (P>0.05) by grazing frequency in the first growing season. The proportion of dead material was greater (P<0.05) in the four week than in the two grazing frequency in the second growing season.

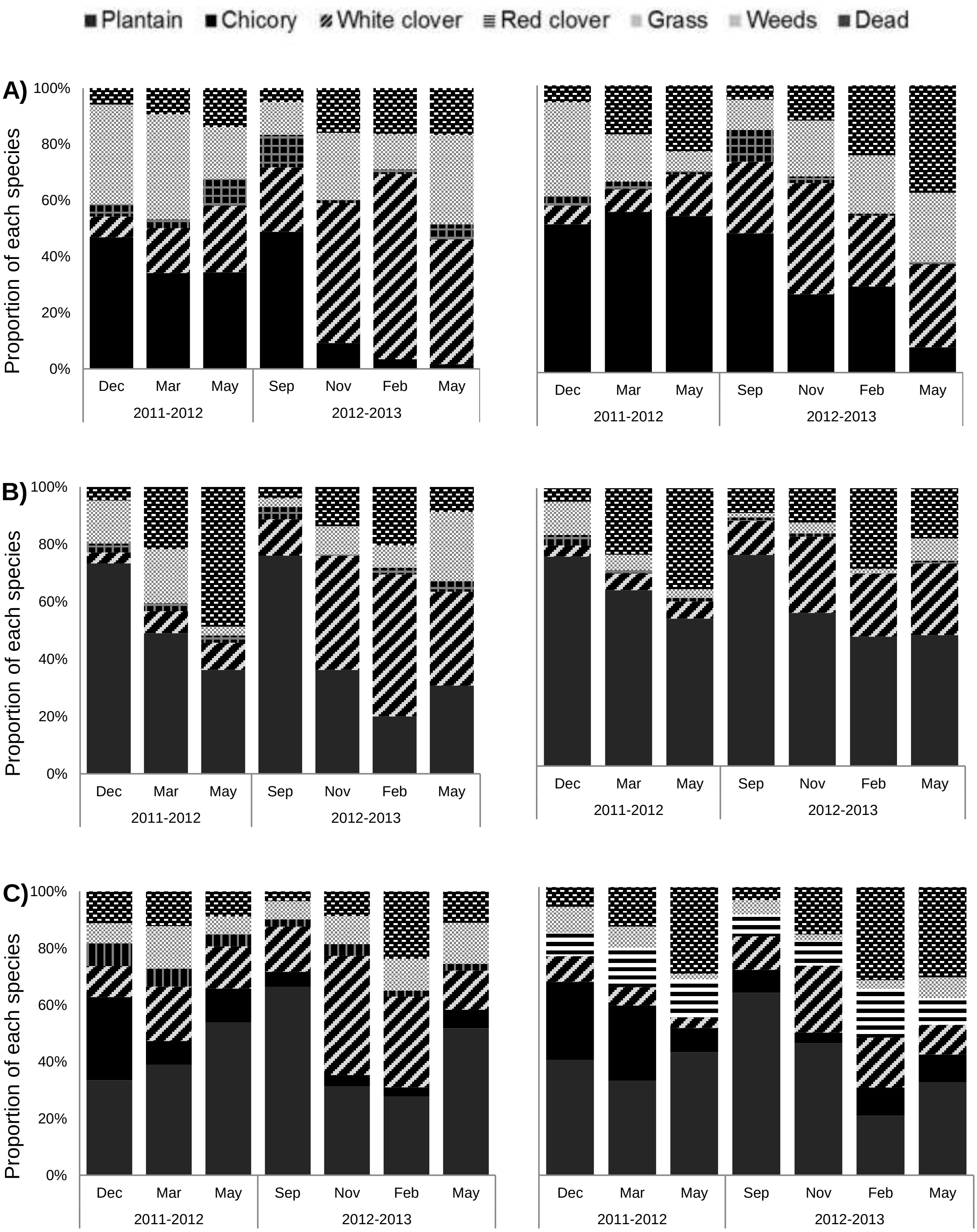

Figure 3-3 Botanical composition (%) in A) chicory, B) plantain and C) the herb-clover mix pastures grazed every two (left) or every four week (right) frequencies throughout the first (2011-2012) and second (2012-2013) growing seasons.

3.4.3 Plant density and plant size

The grazing trial started on December, 2011 with an average plant density in the pure swards of 235 plants/m^2 and 349 plants/m^2 for chicory and plantain, respectively; and 156 plants/m^2 and 258 plants/m^2 for chicory and plantain in the herb-clover mix pasture, respectively. The plant density of plantain was greater ($P<0.05$) than chicory in both pure swards and in the herb-clover mix pasture during both growing seasons. The plant density of chicory and plantain was greater ($P<0.05$) in the pure swards than in the herb-clover mix pasture in both growing seasons (Figure 3-3).

During the first growing season (2011-2012), the plant density of chicory remained constant in the pure swards but decreased by 34% from its initial plant density in the herb-clover mix pasture by the end of the first growing season (May, 2012; Figure 3-4A). During the second growing season (2012-2013), the plant density of chicory declined ($P<0.05$) in both the pure and the herb-clover mix pastures. The decline was greater ($P<0.05$) in the pure swards than in the herb-clover mix pasture, and by November, 2012 there were 64% and 44% decrease in chicory density for the pure and the herb-clover mix pasture, respectively. The plant density of chicory from November, 2012 onwards was similar ($P>0.05$) between the pure and the herb-clover mix pasture and greater than February and May 2013.

Grazing frequency did not affect ($P>0.05$) the plant density of chicory during the first growing season (2011-2012), but during the second growing season (2012-2013), the plant density of chicory was greater ($P<0.05$) in the swards grazed every four weeks compared to those grazed every two weeks in both the pure and herb-clover mix pastures. There were no treatment interactions ($P>0.05$) for the plant density of chicory during the first (2011-2012) and second growing seasons (2012-2013).

Plantain plant density followed a similar pattern in both the pure and the herb-clover mix pastures (Figure 3-4B). During the first growing season (2011-2012), there was an increase ($P<0.05$) in the plant density of plantain at the end (May, 2012) of the first growing season compared to the initial plant density (December, 2011) in both the pure and herb-clover mix pastures.

The second growing season started (August, 2012) with a higher plantain density compared to the plantain density at end (May, 2012) of the first growing season. During the second growing season, plantain densities declined ($P<0.05$) in both the pure and the herb-clover mix pastures. The plantain density in November 2012 declined by 38% and 55% for the pure and the herb-clover mix pasture, respectively, but it was greater than the plantain density in the months of February and May, 2013. The plant density of plantain was not affected ($P>0.05$) by grazing frequency throughout both growing seasons.

The plant size (shoots/plant) of chicory (Figure 3-5A) in the first growing season remained constant (average 1 shoot/plant) regardless of grazing frequency, in both the pure and the herb-clover mix pastures. However, in the second growing season, there was a three-way interaction ($P<0.05$) between pasture type, grazing frequency and month for plant size of chicory (Figure 3-5A). The number of shoots per chicory plant in the pure swards was similar in August and November, 2012 but increased in February, 2013 and then remained constant afterwards when chicory was grazed every four weeks compared to a two weeks frequency. In the second growing season, chicory plants in the herb-clover mix did not develop secondary shoots per plant (average 1 shoot/plant) regardless of the grazing frequency.

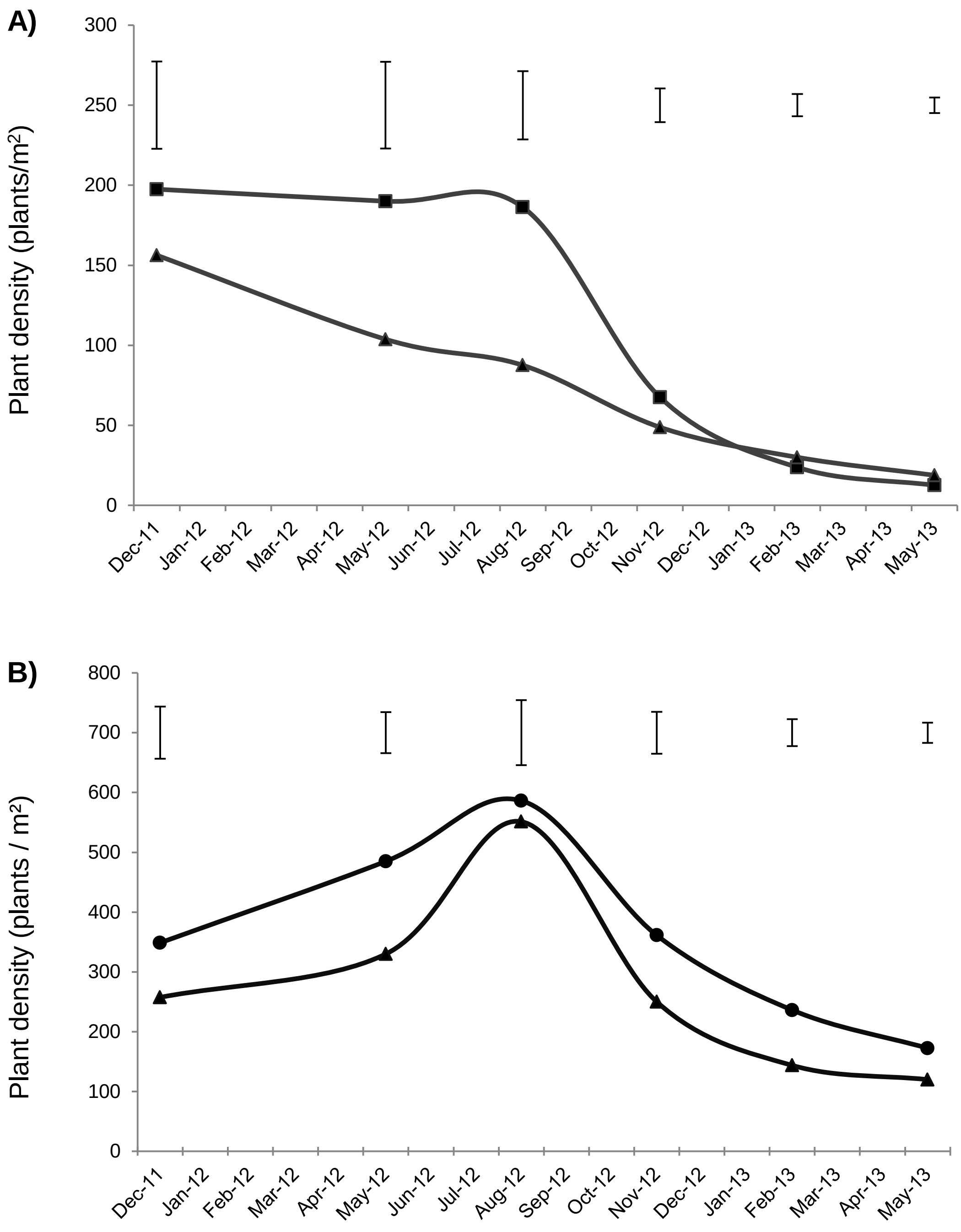

Figure 3-4. Plant density (plants/m^2) of A) chicory in the pure sward (■) and in the herb-clover mix (▲) pasture and of B) plantain in the pure sward (●) and in the herb-clover mix (▲) pasture throughout the first (December, 2011 and May, 2012) and second (August, 2012; November, 2012; February, 2013; May 2013) growing seasons. Vertical bars represent the standard error of the mean (SEM).

The plant size of plantain in the first growing season was affected by a two-way interaction ($P<0.05$) between grazing frequency and month (Figure 3-5B). Plantain plants progressively increased their number of shoots per plant throughout the first growing season when grazed every two weeks but remained constant when grazed every four weeks. During the second growing season, the plant size of plantain was greater ($P<0.05$) when grazed every two weeks when compared to four weeks grazing and increased throughout the growing season. The number of shoots per plant of plantain remained similar from August to November, 2012 and increased in February and May, 2013 in both the pure and herb-clover mix pastures. There were no treatment interactions ($P>0.05$) for plant size of plantain in either growing season.

Chicory plants in the pure swards were monitored monthly during the second (2012-2013) growing season (Figure 3-6). The pure chicory swards grazed every four weeks had a greater ($P<0.05$) herbage mass of chicory (kg DM/ha) compared to those grazed every two weeks from October, 2012 onwards. Chicory grazed every four weeks had a similar herbage mass during the spring months (October to December, 2012) but was lower than in summer (January to March 2013); while the herbage mass from chicory in the swards grazed every two weeks declined during spring (October to December, 2012) and from January, 2013, it remained low until the end of the second growing season.

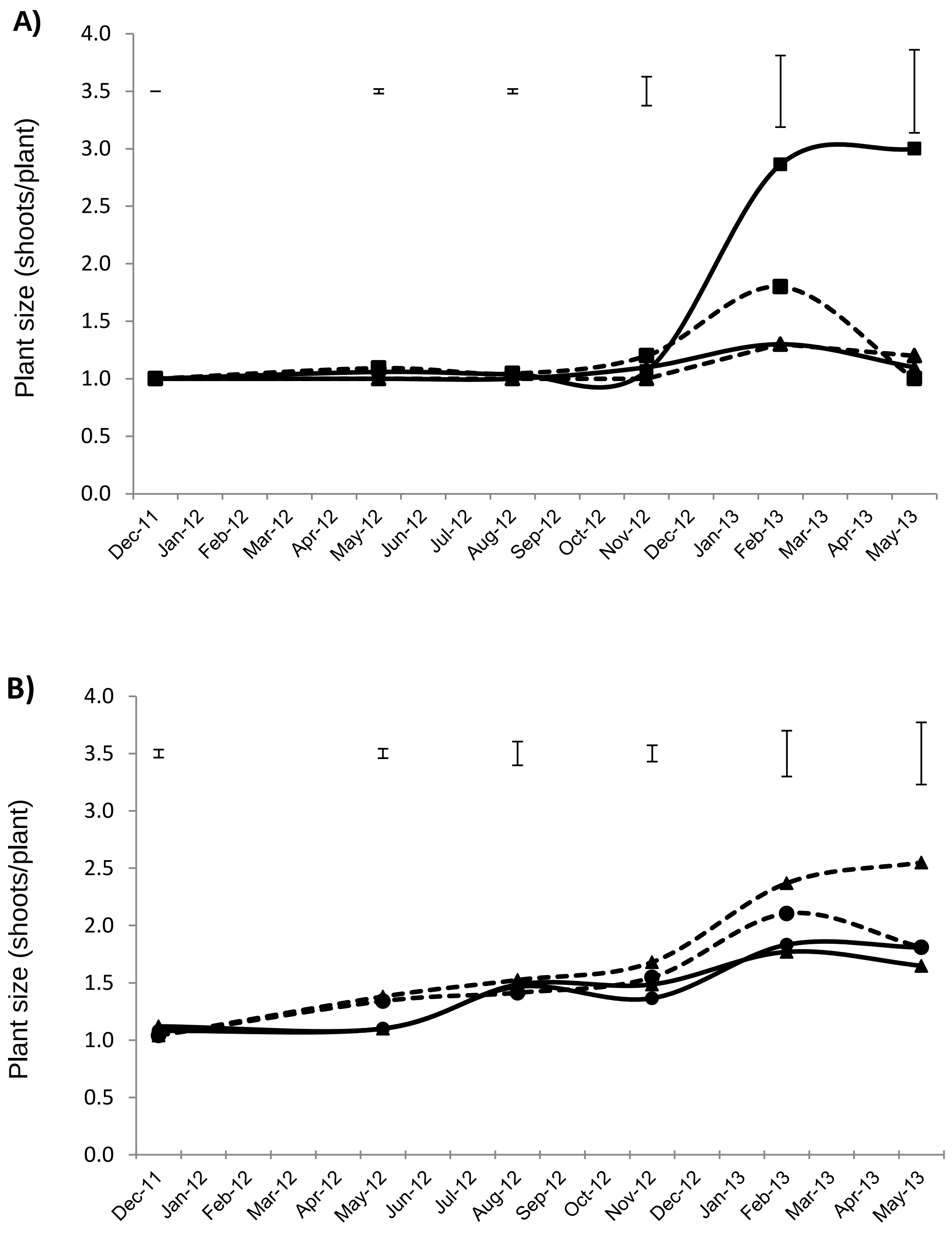

Figure 3-5 Plant size (shoots/plant) of A) chicory plants in the pure sward (■) and in the herb-clover mix (▲) pasture and of B) plantain plants in the pure swards (●) and herb-clover mix pastures (▲) under a grazing frequency of two (dashed lines) and four (solid lines) weeks throughout the first (December, 2011 and May, 2012) and second (August, 2012; November, 2012; February, 2013; May, 2013) growing seasons. Vertical bars represent the standard error of the mean (SEM).

Pure chicory swards grazed every four weeks had a greater ($P<0.05$) shoot density (shoots/m^2) compared to chicory grazed every two weeks (Figure 3-6). Shoot density (shoots/m^2) declined from September, 2012 to November, 2012 in both grazing frequencies; however, in the chicory swards grazed every four weeks the shoot density remained higher from December, 2012 onwards compared to the chicory grazed every two weeks.

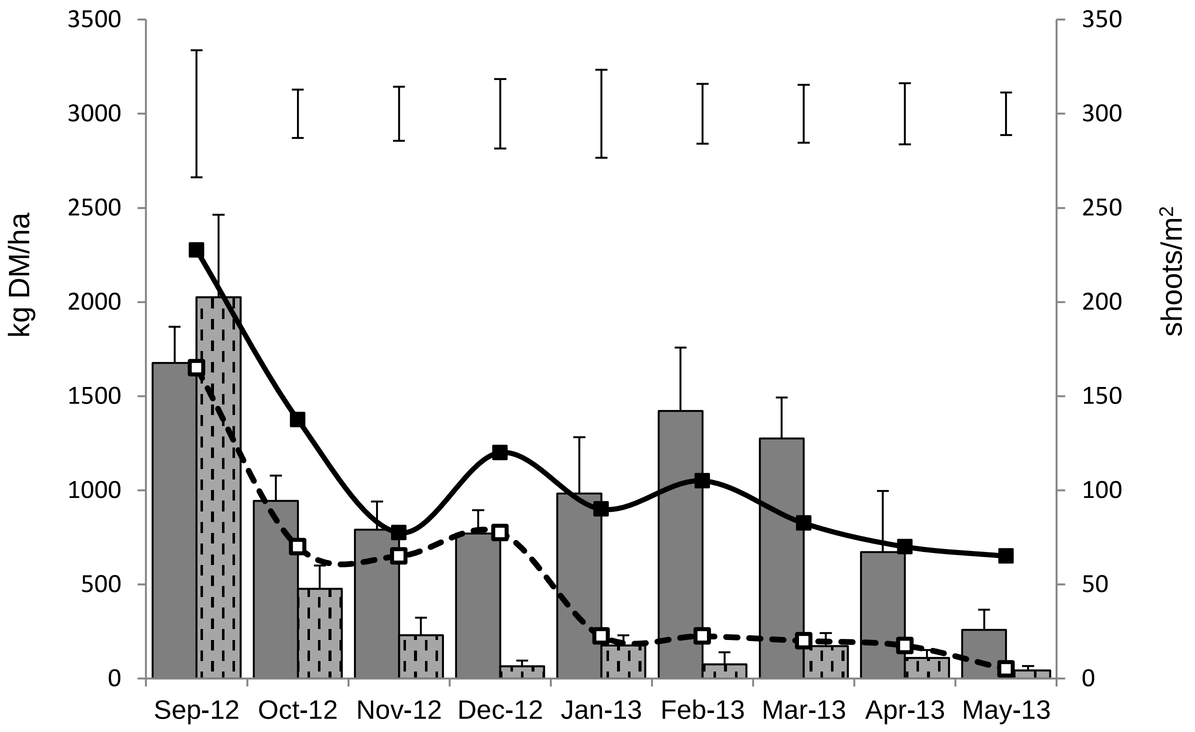

Figure 3-6 Monthly herbage mass contributed by chicory (kg DM/ha; column) in the pure swards grazed every two (dashed vertical) and every four (solid fill) week frequencies and shoot density (shoots/m^2; lines) in the pure chicory swards by grazing every two (dashed lines) and every four (solid lines) week frequencies throughout the second (2012-2013) growing seasons. Vertical bars represent the standard error of the mean (SEM).

3.4.4 Taproot development and carbohydrate reserves

3.4.4.1 Taproot diameter

The taproot diameter for chicory was greater (P<0.05) than plantain throughout both growing seasons. Chicory plants developed greater (P<0.05) taproots in the pure swards than in the herb-clover mix pasture during the first (2001-2012) and second (2012-2013) growing seasons (Table 3-4). Chicory plants in the pure swards developed greater (P<0.05) taproots in the four week than in the two week grazing frequency from the first growing season; but taproot diameter of chicory plants in the herb-clover mix remained similar (P>0.05) irrespective of grazing frequency in both growing seasons (Table 3-4).

In contrast, the taproot diameter of plantain was similar (P>0.05) in both the pure swards and the herb-clover mix pasture and was not affected (P>0.05) by grazing frequency in either growing seasons (Table 3-4). There was neither a two-way interaction (P>0.05) between grazing frequency and month for the taproot diameter of chicory and plantain nor a three-way interaction (P>0.05) between herb species, grazing frequency, and month.

Table 3-4 Taproot diameter (mm) of chicory and plantain (mean ± SEM[1]) in the pure swards and in the herb-clover mix pasture by grazing every two or every four week frequencies during the first (2011-2012) and second (2012-2013) growing seasons.

Species	Pasture type	Grazing frequency	
		2 weeks	4 weeks
First growing season (2011-2012)			
Chicory	pure swards	7.9 ± 0.58 [a]	9.2 ± 0.61 [b]
	herb-clover mix	6.1 ± 0.44 [c]	6.1 ± 0.41 [c]
Plantain	pure swards	3.7 ± 0.09 [d]	3.6 ± 0.15 [d]
	herb-clover mix	3.2 ± 0.12 [d]	3.7 ± 0.15 [d]
Second growing season (2012-2013)			
Chicory	pure swards	12.9 ± 0.84 [a]	15.0 ± 0.84 [b]
	herb-clover mix	8.0 ± 0.54 [c]	9.0 ± 0.58 [c]
Plantain	pure swards	4.5 ± 0.28 [d]	4.4 ± 0.24 [d]
	herb-clover mix	4.5 ± 0.22 [d]	4.5 ± 0.23 [d]

[a, b, c] superscripts indicate values within each growing season that are significantly different (P<0.05)

[1] SEM: standard error of the mean.

3.4.4.2 Carbohydrate of reserves in chicory and plantain roots

The concentration of carbohydrate reserves in the roots of chicory and plantain were monitored during the second growing season (2012-2013). Grazing frequency increased ($P<0.05$) the fructan concentration in the chicory and plantain roots grazed every four weeks (119.6 g/kg DM) compared to those grazed every two weeks (91.8 g/kg DM).

The fructan concentration in chicory and plantain roots was affected for an interaction ($P<0.05$) between pasture type and month (Figure 3-7). In August, 2012, the fructan concentration was greater ($P<0.05$) in the roots of chicory and plantain in the pure swards than in the herb-clover mix, with a fructan concentration of chicory in the herb-clover mix similar ($P>0.05$) to plantain in the pure swards. The fructan concentration in chicory and plantain declined ($P<0.05$) from August, 2012 to November, 2012, with both species having a similar ($P>0.05$) concentration of fructan in their roots in November, 2012. However, chicory roots increased ($P<0.05$) their concentration of fructan in February, 2013 until May, 2013 compared to November, 2012, which was higher ($P<0.05$) in the herb-clover mix than in the pure swards. The fructan concentration in plantain, in contrast, remained similar ($P>0.05$) from November, 2012 to May, 2013 in both the pure and the herb-clover mix (Figure 3-7). There were no interactions ($P>0.05$) between pasture type and grazing frequency or between pasture type, grazing frequency and month for the fructan concentration in chicory and plantain roots.

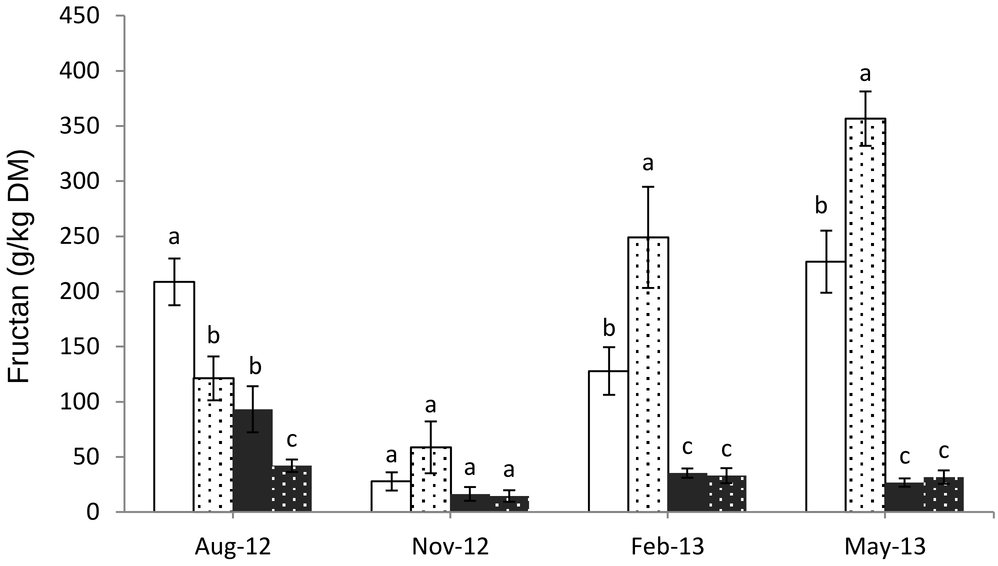

Figure 3-7 Fructan concentration (g/kg DM) in the roots of chicory (white) and plantain (black) in the pure swards (solid fill) and in the herb-clover mix (dashed horizontal) pasture throughout the second (2012-2013) growing season. Vertical bars represent the standard error of the mean (SEM). [a, b, c] letters indicate values that are significant different (P<0.05) in each month.

The concentrations of the reducing sugars, glucose, fructose, and sucrose was greater (P<0.05) in roots of chicory than in plantain regardless of grazing frequency (Table 3-5). There was no difference (P>0.05) in the concentrations of the reducing sugars in root chicory and plantain in pure sward and herb-clover mix. The concentration of fructose and glucose changed (P<0.05) between months in both chicory and plantain, whereas the concentration of sucrose remained constant (P>0.05) between months in both herbs (Table 3-5).

There was an interaction (P<0.05) between grazing frequency and month only for the concentration of fructose (data not shown). The fructose concentration in August, 2012 and November, 2012 was greater (P>0.05) when grazed every four weeks rather than when grazed every two weeks, but in February, 2012 and May, 2013 the fructose concentration was similar (P>0.05) in both grazing frequencies. There was no two-way interaction (P>0.05) between herb species and grazing frequency nor a three-way interaction (P>0.05) between herb species, grazing frequency and month for the concentration of glucose, fructose and sucrose.

Table 3-5 Concentration (mg/g DM) of fructose, glucose and sucrose in roots of chicory and plantain during the second (2012-2013) growing season.

	Chicory			**Plantain**		
Month	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
Aug - 12	89.7[a]	14.2[a]	31.7	20.0[a]	15.9[a]	1.2
Nov - 12	70.6[b]	15.9[a]	24.5	14.2[b]	6.9[c]	1.7
Feb - 13	16.3[c]	16.8[a]	17.6	3.5[c]	11.1[b]	1.9
May - 13	22.8[c]	9.4[b]	17.3	11.3[b]	12.7[b]	2.5
SEM[1]	1.27	5.21	4.18	0.70	1.54	0.32

[a, b, c] superscripts indicate values within column that are significantly (P<0.05) different

[1] SEM: standard error of the mean.

3.5 DISCUSSION

3.5.1 Herbage Production

Plantain and the herb-clover mix pastures yielded more than chicory and perennial ryegrass/white clover pastures in the first (2011-2012) and second (2012-2013) growing seasons (Table 3-3). The greater production of plantain than chicory was within the range of other research conducted in New Zealand (Glassey et al., 2013; Lee et al., 2015; Li et al., 1997b; Powell et al., 2007). Whereas there have been few studies reporting DM production for herb-clover mix pastures over whole growing seasons, in New Zealand. A recent study reported a DM production for herb-clover mix pastures (9 - 11 t DM/ha) that was lower than in the current work (Cranston, 2014). A greater DM production was expected for the herb-clover mix pasture compared to pure chicory or plantain swards (Goh and Bruce, 2005). However, the advantage of combining chicory, plantain, red, and white clover in an herb-clover mix pasture is that it provides a pasture with a longer growing season compared to any of the individual species grown alone (Kemp et al., 2010). The herb-clover mix improved DM distribution over the summer/autumn period in comparison with typical perennial ryegrass pastures (Jacobs et al., 2006; Moorhead and Piggot, 2009). However, in this study the herb-clover mix pasture showed an annual DM production and herbage mass pre-grazing with values similar to pure plantain swards, throughout both growing seasons (Table 3-3; Figure 3-1).

Chicory and plantain sown as pure swards or in the herb-clover mix pasture showed that both herbs established well in all field plots, supported by the high germination rate recorded for the seed and by the initial plant density in the pastures (Figure3-3). The plant density of plantain and chicory was in agreement with the study of (Lee et al., 2015).conducted in New Zealand (260 - 330 plantain plants/m^2 and 170 - 200 chicory plants/m^2). The sowing rates used in this study were based on the weight of a thousand seeds to ensure a similar seed number per unit area (Glassey et al., 2013; Powell et al., 2007). However, the initial plant density showed that approximately 98% and 60% of the viable seed sown in plantain and chicory pastures, respectively, were successfully established. Consequently, plantain maintained a greater plant density than chicory (Figure 3-3), consistent with previous work in which both herbs were evaluated (Glassey et al., 2013; Labreveux et al., 2004; Lee et al., 2015; Powell et al., 2007; Sanderson et al., 2003). The lower chicory plant density might be associated with a lower persistence after sowing.

The annual DM production of the pasture treatments did not differ between both the two or four week grazing frequencies in either growing seasons (Table 3-3), This is contrary to previous findings, in New Zealand, chicory pastures defoliated at eight or four week intervals have accumulated higher herbage mass (stem and leave) than those defoliated at one or two week intervals (Clark et al., 1990; Li et al., 1997b). Similarly, in the USA, defoliation of chicory at intervals of four or six weeks increased DM production compared to two week intervals (Volesky, 1996), or in five week compared to a three week interval with chicory and plantain (Labreveux et al., 2004; Sanderson et al., 2003). Recently, Lee et al. (2015) reported that chicory defoliated at 35 or 55 cm and plantain at 35 or 45 cm of extended leaf height (ELH) yielded more than those plants that defoliated at 15 or 25 cm of ELH. In this study grazing frequency altered the contribution of the sown herbs in the pastures (Figure 3-3). Grazing every two weeks reduced the proportion of chicory and plantain in the pure swards, and, hence, most of the DM came from white clover and weeds compared to grazing every four weeks, in both growing seasons (Figure 3-3A and B).

3.5.2 Pasture persistence

Persistence of pasture can be defined as the number years in which a pasture performs near potential DM production. Poor persistence occurs when the sown species is replaced by weed through a decline in the plant density of the sown species decreasing the DM contribution of the sown species (Parsons et al., 1991; Tozer et al., 2011). In this study, the decline in the plant density of chicory and plantain occurred from their second growing season (Figure 3-4). The greater decline in plant density was during late spring (November, 2012) and summer (February, 2013), with chicory (44 to 64%) having a higher decline in plant density than plantain (38 to 55%). Spring has been described as a critical period in the management of chicory, in which chicory has high growth but also has a high plant death rate (Li and Kemp, 2005). The major cause of plant death appeared to be related to the inability of some chicory plants to recover after grazing due to low root reserves after winter (Li et al., 1997b). However, these results also suggested that dairy cows, as heavy animals, may have potentially produced a greater treading damage in chicory plants than in plantain plants. However, it is clear that the decline in plant density was unavoidable for both herbs under grazing conditions.

Chicory in the pure swards retained its plant density during the first growing season, regardless of grazing every two or every four weeks (Figure 3-3A). Several studies have reported that chicory density decreased from the establishment year regardless of defoliation management (Glassey et al., 2013; Labreveux et al., 2004; Lee et al., 2015; Li et al., 1997b; c; Powell et al., 2007; Sanderson et al., 2003). Under grazing conditions during a first growing season, a decrease of 35% of chicory density has been reported when grazing with sheep (Li et al., 1997a). However, greater reductions (of 45%) of chicory plants have occurred when swards were established with herbicide spraying and direct drilling and then grazed by dairy cows, as in this study (Glassey et al., 2013). This result might be related to the grazing management during the first growing season, where chicory plots were grazed until April, 2012 due to the low growth observed in chicory plants at this time. However, the plant density of chicory and plantain was evaluated on May, 2012. This management decision may have provided the opportunity for the growth of chicory plants by the time of plant density sampling.

Grazing management is an important key to pasture persistence. In the current study, grazing frequency affected chicory plant density, but did not have an effect for plantain density in either growing season. Most of the decrease in plant density of chicory was during late spring and summer as reported previously by others (Li et al., 1994 and Li et al., 1997a; Lee et al., 2015). The net herbage mass of chicory has been reported to remain stable until its plant density declines to less than half of the original plant density (Li et al., 1997b). However, during late spring, chicory swards grazed under both grazing frequencies still maintained a plant density above the optimal (>50 plants/m^2) stated for a highly productive chicory pastures (Li and Kemp, 2005).

Nevertheless, grazing chicory every two weeks caused a stronger impact on the plant density of chicory compared to being grazed every four weeks. The decrease in chicory density in the two week frequency was accompanied by an increase in weeds, predominantly white clover (Figure 3-2). This weed invasion was a consequence of the decline in plant density but the weed competition could have also hastened stand decline (Sanderson et al., 2003; Teixeira et al., 2007). Chicory grazed every two weeks failed to persist from January, 2013 due to the fact that at this point, chicory swards had deteriorated to produce half of its maximum herbage mass (<25 plants/m^2 and <150 shoots/m^2) (Li and Kemp, 2005). When the plant density of chicory decline below 25 plants/m^2, the increase in shoots per plant cannot compensate for the decreased herbage production due to the decline in plant density. Besides, in this study, chicory grazed every two weeks was not able to develop extra shoots during its second growing season and plant size started to decrease in the two week frequency treatments from January, 2013. Smaller plants of chicory and lucerne (*Medicago sativa* L.) are possibly more susceptible to death after grazing than those that survive (Leach and Ratcliff, 1979; Li et al., 1997c).

Chicory grazed every four weeks maintained a higher herbage DM contribution from chicory in the swards and a greater shoot density than those grazed every two weeks (Figure 3-4). Chicory grazed every four weeks showed that the decline in plants in late spring was compensated by an increase in shoots per plant (Li et al., 1994). Plant size increased throughout the season, especially from late spring to summer, which compensated for the decrease in plant density (Li et al., 1997b; Teixeira et al., 2007). Shoot density was more stable from January, 2012 onwards in response to the decline in plant density. However, chicory grazed every four weeks had a plant density lower than the optimal recommended (<50 plants/m^2) by March, 2013. Chicory is very sensitive to late autumn grazing in terms of plant density and it is recommended to avoid late autumn grazing for the persistence of chicory in the following growing season (Li and Kemp, 2005). In this study, chicory was grazed until the end of May, 2013 and this resulted in a decreased chicory contribution in the swards by the end of the second growing season (May 2013). This suggests that chicory under a four week grazing frequency would likely not persist for a third growing season.

Plantain was more flexible in its response to grazing frequency than chicory. Grazing plantain under managements with either different frequencies (Ayala et al., 2011a; Labreveux et al., 2004), ELH from 15 to 45 cm (Lee et al., 2015), or intensity with residual heights ranging from 20-120 mm have shown do not affect plantain density (Lee et al., 2015; Ayala et al., 2011a; Labreveux et al., 2004). In this study, the plant density of plantain in the first growing season (2011-2012) increased by the end (May, 2012) of this season (Figure 3-3B). This is contrary to the findings of Powell et al. (2007) and Glassey et al. (2013) who reported that plantain density declined after the first year of grazing, similar to the pattern of chicory. Nevertheless, Lee et al. (2015) recently reported that plantain density remained constant during the first year when defoliating at 15, 25, 35 or 55 cm of ELH, and plantain density declined after the second growing season, similar to the findings of this study.

In addition, the increased plantain density from May, 2012 to August, 2013 is likely attributable in part to seedling recruitment of the plant, as observed by Ayala et al. (2011b), which is uncommon in chicory (Li et al., 1997a). Reproductive stems and older mature leaves of plantain were avoided by sheep (Ayala et al., 2011b; Ivins, 1952), and as result, plantain may be able to reseed in pasture (Derrick et al., 1993). Similarly, when an infrequent (six week interval) and lax (10 cm residual height) grazing strategy was applied, swards retained a higher plantain population during autumn (Ayala et al., 2011b). Therefore, plantain might persist longer if reseeding from natural regeneration is promoted (Nie et al., 2008) and under appropriate management of plantain ensuring seedling recruitment will lead to high productive and more persistence pastures (Neal et al., 2007).

Plantain plants responded to grazing frequency by increasing the number of shoots per plant when swards were grazed every two weeks (Figure 3-5B). The increased plant size caused by grazing every two weeks might be due to changes in the amount of light penetrating the shoot base, influencing shoot appearance as occurs in perennial ryegrass (Langer et al., 1964). Perennial ryegrass uses its tillering ability to adapt to defoliation regimens; consequently, plantain shoot size and density may have the same inverse relationship (Langer et al, 1964) meaning that as the herbage mass increased, tillers are fewer but larger, and leaf area index (LAI) and herbage accumulation remain constant (Matthew et al., 1995). Thus, any effect of management practice on the sward could be masked by size/density compensation (Yoda et al., 1963).

3.5.3 Root development and carbohydrate reserve

Chicory plants developed greater taproot diameter and stored higher concentrations of carbohydrate reserves in their roots than plantain (Table 3-4 and 3-5). Both herbs, as tap-rooted plants, store carbohydrate reserves in their roots for regrowth after defoliation (Kemp et al., 2010), but chicory has a dominant primary root and a greater root mass than plantain (Powell et al., 2007; Sanderson and Elwinger, 2000). For chicory, fructan is the major carbohydrate reserve, hence, chicory roots accumulated higher amounts of total reducing sugars than plantain roots (Li et al., 1997a). These carbohydrate reserves are used as an energy source to initiate new growth until photosynthesis is sufficient to sustain plant respiration (White, 1973). Chicory plants after defoliation first form a large leaf area using the carbohydrates reserves in their roots systems (Li et al., 1997a). Nevertheless, the lower concentration of carbohydrate reserves in plantain roots compared to chicory may suggest that plantain would be more dependent on residual leaf area than root carbohydrate for regrowth, similar to perennial ryegrass (Fulkerson and Donaghy, 2001). Smaller roots and the lower concentration of root reserves in plantain compared to chicory roots suggest that plantain regrowth after defoliation might not depend on its roots carbohydrate reserves to the same extent as chicory.

The effect of grazing frequency caused both herb species to accumulate more fructan when grazed every four weeks than when grazed every two weeks. Both herbs had greater concentration of fructose during spring (August and November, 2012) when swards where grazed every four weeks compared to those grazed every two weeks. Hence, both chicory and plantain require longer grazing intervals, greater than two weeks, to replenish their root carbohydrate reserves. Similarly, other tap-rooted plants, such as lucerne and red clover, have shown that increasing defoliation frequency depleted their roots carbohydrate reserves (Smith et al., 1989; White, 1973). In this study, chicory grazed at two week intervals did not develop large taproots, and plants with smaller taproots are more susceptible to death after defoliation (Li et al., 1997a). The plants grazed every two weeks may begin to grow smaller leaves, and reduce root size, and shoot initiation. By doing this the plant reduces the amount of carbohydrates utilised for plant growth and lost during respiration, which may able to the plant to survive subsequent defoliations (Lee et al., 2015). A grazing schedule every two weeks on chicory plants might kill plants by depleting their root carbohydrate reserves compared to grazing chicory every four weeks.

In contrast, the taproot diameter of plantain was not affected by grazing frequency. However, plantain plants grazed frequently (every two weeks) seemed to allocate more energy to shoot growth than when grazing every four weeks. This response might suggest that plantain increased its number of shoots per plant to maintain a leaf area for photosynthesis, and would be more dependent on a residual leaf area. The major storage site for water soluble carbohydrate (WSC) in temperate grasses is the stubble with the roots playing only a minor role (Fulkerson and Slack, 1994). Therefore, plantain could be defoliated more frequently by leaving a residual leaf area.

3.6 CONCLUSIONS

Plantain and herb-clover mix pastures were more productive and persisted for the two growing seasons compared to chicory pasture. Grazing frequency affected the proportion of chicory and plantain in the pure swards; while the herb-clover mix maintained a higher proportion of the sown species regardless of the grazing frequency. Grazing every two weeks was detrimental to the persistence of chicory. This grazing frequency caused a stronger loss of chicory plants which also did not develop large taproots compared to those grazed every four week frequency. Plantain, in the pure sward and herb-clover mix, was more durable in its response to grazing frequency. So, farmers should graze chicory at intervals of at least four weeks and probably longer for a pasture to persist for at least two complete growing seasons. Plantain and the herb-clover mix can be grazed either every two or every four weeks and persist for at least two growing seasons.

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4 NUTRITIVE VALUE OF HERB PASTURES GRAZED AT TWO FREQUENCIES THROUGHTOUT TWO GROWING SEASONS

4.1 ABSTRACT

Herb pastures, such as chicory (*Cichorium intybus* L.) or plantain (*Plantago lanceolata* L.), and herb-clover mix pastures can produce herbage of high quality during summer; however, there is limited information on the effect of grazing management in dairy systems on the nutritive value of these pastures throughout a complete growing season. This study aimed to determine the effect of grazing frequency on the nutritive value of chicory, plantain, and herb-clover mix pastures during spring, summer, and autumn throughout two growing seasons. A grazing experiment carried out between December, 2011 and May, 2013 evaluated and compared the following pasture types: (i) chicory; (ii) plantain; and (iii) a herb-clover mix, containing chicory, plantain, red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.). These pastures were grazed every two and every four weeks by dairy cows. Hand-plucked samples from the pastures were taken in spring (early and late), summer, and autumn during the two growing seasons (2011-2012 and 2012-2013). The samples were analysed for dry matter (DM), metabolisable energy (ME), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, hot water soluble carbohydrates (HWSC), and ash. Chicory, plantain, and herb-clover mix pastures had optimum CP (>160-180 g CP/kg) and NDF concentrations (<350 g NDF/kg DM) for lactating dairy cows. The ME concentration of chicory was stable throughout the different seasons; whilst plantain and the herb-clover mix pasture had a lower ME concentration in late spring and summer associated with an increased NDF concentration. Grazing every two weeks increased the CP concentration and decreased the NDF concentration compared to grazing every four weeks. Consequently, grazing plantain and the herb-clover mix every two weeks during late spring and summer may be a means to improve their nutritive value.

Keywords: Chicory, plantain, herb-clover mix, nutritive value, grazing frequency

4.2 INTRODUCTION

Herbage production and quality of perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) pastures often become limited in summer and autumn leading to feed deficits and lower animal production (Li and Kemp, 2005; Sanderson et al., 2003). To alleviate this feed deficit, the perennial herbs chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) can be used to provide large amounts of high quality forage to supplement dairy cows during the summer/autumn period (Lee et al., 2015; Powell et al., 2007). Both herbs can be sown in combination with red (*T. pratense* L.) and white clover in a mixed sward providing a more productive and high quality pasture, with the advantage of prolonging the growing season compared with any of those species by themselves (Kemp et al., 2010).

Feeding chicory or plantain has led to higher growth rates in both lambs and deer during summer and autumn when compared to perennial ryegrass (Hoskin et al., 1995; Hoskin et al., 2006; Kusmartono et al., 1997; Moorehead et al., 2002). Both herbs have been shown to increase milk solids production in dairy cows when offered as a supplement to pasture when compared with cows solely fed pasture in summer and autumn (Minnee et al., 2012; Waugh et al., 1998). Recent research on mixed swards containing chicory, plantain, red, and white clover has shown improved ewe milk production, increasing lamb weaning weights, and higher lamb growth rates post-weaning when compared to perennial ryegrass (Golding et al., 2011; Hutton et al., 2011; Kemp et al., 2010; Kenyon et al., 2010). This demonstrates that herb-clover mix pastures may have the potential to improve milk production in dairy systems.

Compared to perennial ryegrass, both herbs have higher concentrations of water soluble carbohydrates (WSC) and pectin (readily fermentable carbohydrate; RFC), but lower neutral detergent fibre (NDF) (Barry, 1998; Fraser and Rowarth, 1996; Swainson and Hoskin, 2006). The herbage produced by chicory and plantain contain less cellulose and hemicellulose (structural carbohydrate; SC) but more lignin than perennial ryegrass (Kusmartono et al., 1997; Swainson and Hoskin, 2006). The lower NDF concentration (Fraser and Rowarth, 1996) and the high ratio of RFC to SC results in chicory and plantain have greater degradation rates (0.26 and 0.25 %/h, respectively) than perennial ryegrass pastures (0.11 %/h) (Barry, 1998; Burke et al., 2000), allowing greater dry matter intake (DMI) and thus increased animal production (Li and Kemp, 2005). Consequently, compared to perennial ryegrass/white clover pastures, the herbage produced from herb and herb-clover mix pastures have high nutritive value for dairy cows.

The majority of the studies mentioned above have compared the nutritive value of chicory or plantain with perennial ryegrass from pastures grazed with either lamb or deer (Hoskin et al., 1995; Hoskin et al., 2006; Kusmartono et al., 1997; Moorehead et al., 2002) or under mowing conditions (Lee et al., 2015; Sanderson et al., 2003). There is, however limited information on the nutritive value of chicory and plantain and herb-clover mix pastures under grazing management with dairy cows, and the effect that different grazing frequencies may have on the nutritive value of these pastures. The objective of this study was to determine and compare if plantain nutritive value is lower compare chicory and herb-clover mix pastures in spring (early and late), summer, and autumn, when grazed every two and four weeks with dairy cows throughout two consecutive growing seasons.

4.3 MATERIAL AND METHODS

This study reports the nutritive value from herbage produced for chicory, plantain, and herb-clover mix pastures during spring (early and late), summer, and autumn grazed with lactating dairy cows every two and every four week grazing frequencies, throughout two consecutive growing seasons (2011-2012; 2012-2013).

4.3.1 Experimental design

A grazing experiment was conducted at the No. 1 Dairy Farm, Massey University, Palmerston North, New Zealand from December, 2011 to May, 2013 to evaluate three pasture treatments: (i) chicory (cv. 'Grassland Choice'); (ii) plantain (cv. 'Ceres Tonic'); and (iii) an herb-clover mix containing chicory, plantain, red clover (cv. 'Sensation'), and white clover (cv. 'Emerald' and 'Bounty'). The pastures were managed under two grazing frequencies, two or four week intervals, for two growing seasons (2011-2012 and 2012-2013) with lactating dairy cows. Pastures were established in October, 2011 in a randomized complete block (n=5) design with a factorial arrangement of the pasture treatments (plot area of 300 m^2). A perennial ryegrass/white clover pasture was also included as a benchmark and grazed under New Zealand best practice management, whereby the perennial ryegrass/white clover pasture was grazed when pasture had a pre-grazing herbage mass within the range of 2500 - 3000 kg DM/ha. Complete details of the experimental design, pasture treatments and grazing management are reported in Chapter 3.

4.3.2 Herbage measurements for nutritive value

The nutritive value of the pasture treatments and perennial ryegrass/white clover pasture was evaluated during the first growing season (2011-2012) in: (i) late spring (December, 2011); (ii) summer (February, 2012); and (iii) autumn (May, 2012). During the second growing season (2012-2013) the nutritive value was assessed in: (i) early spring (August, 2012); (ii) late spring (December, 2012); (iii) summer (February, 2013); and (iii) autumn (May, 2013). One grab sample (100 g fresh weight) of the pasture treatments and perennial ryegrass/white clover pasture was taken pre-grazing in each plot (five samples per pasture). The herbage from the pastures was taken by hand plucked samples from the grazed horizon (> 7 cm) by walking at random within each plot. All samples were stored at -20 °C until laboratory analysis of nutritive value.

4.3.3 Laboratory analysis

Prior to analysis, samples were freeze-dried and ground to pass through a 1 mm diameter sieve. Total dry matter (DM) was determined by drying the samples at 105 °C in a conventional oven (AOAC, 2000); method 930.15, 925.10) and organic matter (OM) by ashing the samples for 16 h at 550 °C in a furnace (AOAC, 2000; method 942.05). Total nitrogen (N) was determined by combustion using a Leco analyser (AOAC, 2000; method 968.06), and crude protein (CP) was calculated multiplying the N content by 6.25. NDF, acid detergent fibre (ADF) and lignin were analysed in a Tecator Fibertec System using the detergent procedures (Robertson and Van Soest, 1981). Cellulose was calculated as ADF less lignin, and hemicellulose as NDF less ADF. Hot water soluble carbohydrates (HWSC) were measured by the reducing sugar method (Nelson, 1944). Organic matter digestibility (OMD) was determined and hence metabolisable energy (ME) predicted by following the Roughan and Holland (1977) method.

4.3.4 Statistical analysis

All data were analysed using PROC MIXED procedure of SAS (SAS Institute, 2009). The model included the fixed effects of pasture treatment (chicory, plantain, and herb-clover mix), grazing frequency (two and four weeks), season (early and late spring, summer, autumn) and their interactions and block treated as a random effect. The first and second growing seasons were analysed separately due to there was no early spring sampling in the first growing season. Perennial ryegrass/white clover pasture was not included in the statistical analyses as plots were not allocated randomly and its grazing management did not use the every two or four week frequencies as used in the pasture treatments. All mean values of perennial ryegrass are reported as benchmark in each growing season.

4.4 RESULTS

The nutritive value of the pasture treatments was different between pastures and seasons for all the components analyses, in both growing seasons. Grazing frequency affected (P<0.0) CP and HWSC concentration in the first growing season, and CP and ash content during the second growing season. There were two-way interaction (P<0.05) between grazing frequency and seasons in the first growing season (CP, cellulose and ash) and in the second growing season (CP, NDF, ADF, and ash concentration). There was no interaction between herb pastures and grazing frequency nor triple interaction between herb pastures, grazing frequency, and season.

4.4.1 Dry matter

During the first growing season (2011-2012), in late spring and summer, the DM concentration (g DM/kg wet weight) in plantain was greater (P<0.05) than in chicory and the herb-clover mix; whereas, in autumn, the chicory, plantain and the herb-clover mix had a similar (P>0.05) DM concentration (Figure 4-1A; 2011-2012). During the second growing season (2012-2013), in early spring, the DM concentration was higher in plantain than chicory and the herb-clover mix; however in late spring, summer, and autumn, plantain and the herb-clover mix had a greater DM concentration than chicory (Figure 4-1A; 2012-2013). Perennial ryegrass/white clover pasture had a DM concentration numerically greater than the herbs and herb-clover mix pastures at each season during both growing seasons (Figure 4-1A). Grazing frequency of two and four weeks had no effect (P>0.05) on the DM concentration of any of the pasture treatments, nor was there a two-way interaction (P>0.05) between grazing frequency and seasons.

4.4.2 Metabolisable energy

The ME content (MJ ME/kg DM) of the herbs and herb-clover mix was affected for an interaction ($P<0.05$) between pasture treatment and season in both growing seasons. In the first growing season (Figure 4-1B; 2011-2012), chicory had a greater ($P<0.05$) ME content in late spring and summer (12.0 and 11.8 MJ ME/kg DM, respectively) than plantain (11.7 and 11.1 MJ ME/kg DM, respectively) and the herb-clover mix (11.7 and 11.4 MJ ME/kg DM, respectively). However in autumn, the ME content of plantain (12.0 MJ ME/kg DM) was greater ($P<0.05$) than chicory and the herb-clover mix (11.6 MJ ME/kg DM) pastures (Figure 4-1B; 2011-2012). During the second growing season, the ME content of chicory in early spring, late spring, and summer was greater ($P<0.05$) than plantain and the herb-clover mix, but in autumn the ME content was similar ($P>0.05$) between chicory, plantain and the herb-clover mix (Figure 4-1B; 2012-2013). Grazing frequency (two versus four weeks) had no effect ($P>0.05$) on the ME content of the pasture treatments and no interactions ($P>0.05$) between grazing frequency and season nor pasture and grazing frequency were detected.

Perennial ryegrass/white clover had a ME content numerically lower than the herbs and herb-clover mix pastures in late spring (11 MJ ME/kg DM) and summer (10.7 MJ ME/kg DM), but increased in autumn (12.1 MJ ME/kg DM) to a value similar to plantain which was greater than chicory and the herb-clover mix. During the second growing season, the ME content in perennial ryegrass/white clover was numerically lower than the pasture treatments in early spring (11.1 MJ ME/kg DM), summer (11 MJ ME/kg DM), and autumn (11.5 MJ ME/kg DM). However, in late spring (10.8 MJ ME/kg DM) the ME content of perennial ryegrass/white clover was similar to plantain and the herb-clover mix which was lower than chicory.

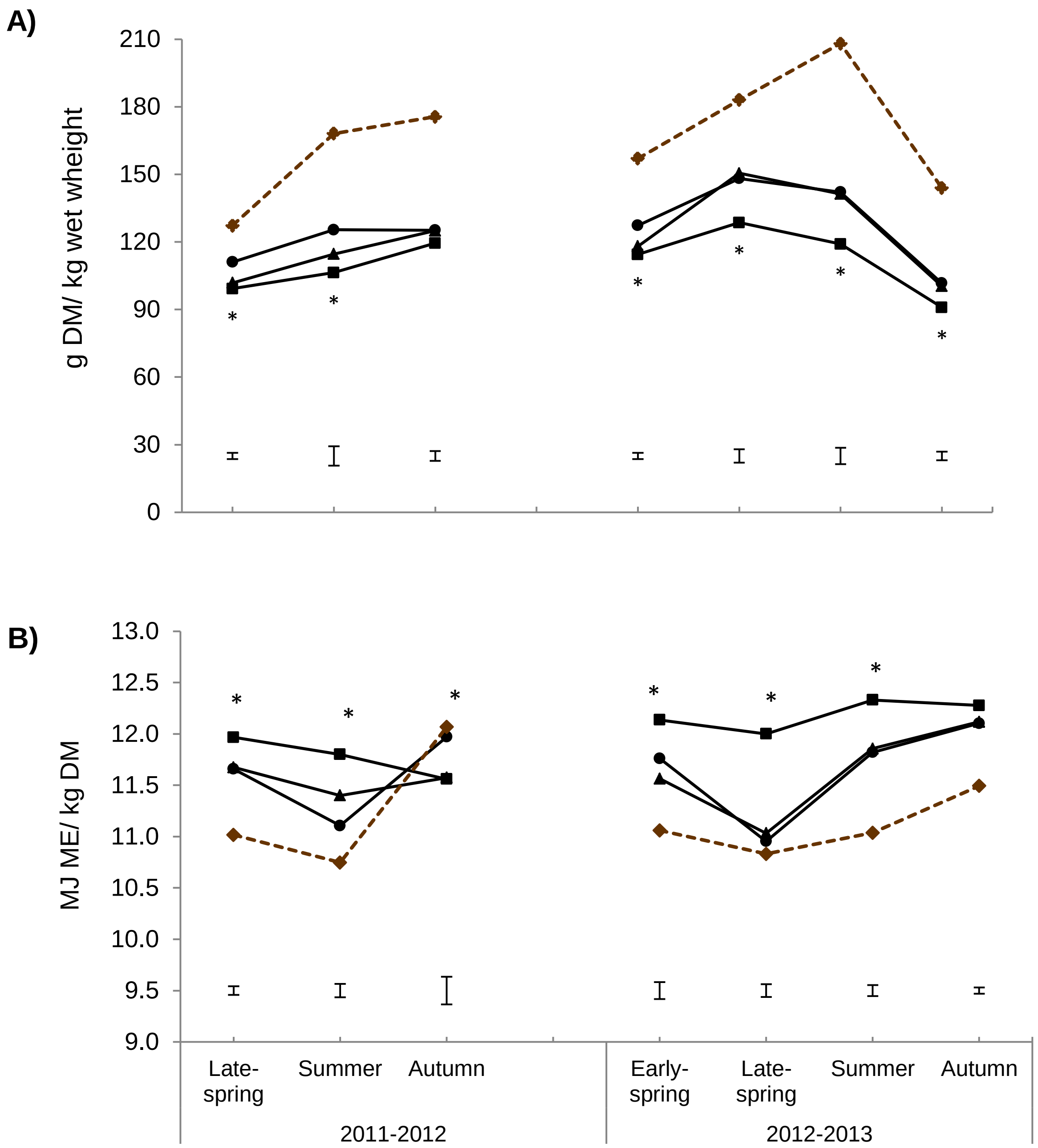

Figure 4-1 Seasonal concentration of A) dry matter (DM) and B) metabolisable energy (ME) in the chicory (■), plantain (●), and herb-clover mix (▲) pasture treatments and in the perennial ryegrass/white clover pasture (♦ dashed lines) during the first (2011-2012) and the second (2012-2013) growing season. Vertical bars represent the standard error of the mean (SEM). * indicate values that significantly differ (P<0.05) between chicory, plantain and herb-clover mix in each season.

4.4.3 Crude protein

During the first growing season (2011-2012), both chicory and the herb-clover mix pasture in late spring (196 g CP/kg DM) and summer (210 g CP/kg DM) had similar (P>0.05) CP concentrations that were greater (P<0.05) than plantain (167 and 174 g CP/ kg DM in late spring and summer, respectively) (Figure 4-2A). All pastures increased their CP concentration in autumn, but the CP concentration of chicory (260 g CP/kg DM) was greater (P<0.05) than that of plantain (226 g CP/kg DM) and the herb-clover mix (241 g CP/kg DM) (Figure 4-2A). During the second growing season (2012-2013), the CP concentration of chicory in early spring (301 g CP/kg DM) was greater (P<0.05) than the herb-clover (249 g CP/kg DM) mix and plantain (211 g CP/kg DM). However, chicory and the herb-clover mix had a similar (P>0.05) CP concentration during late spring (242 g CP/kg DM), summer (262 g CP/kg DM), and autumn (327 g CP/kg DM), which was greater (P<0.05) than plantain (222, 242, and 302 g CP/kg DM in late spring, summer, and autumn, respectively) (Figure 4-2A).

The grazing frequency of two weeks increased (P<0.05) the CP concentration of the herbs and the herb-clover mix pastures compared to the four week grazing frequency in both growing seasons (Figure 4-2B). During the first growing season, in late spring and autumn, the CP concentration did not differ (P>0.05) between grazing frequencies; however, during summer, the herbs and herb-clover mix pastures grazed every two weeks had a CP concentration that was 19 % greater (P<0.05) than those grazed every four weeks (Figure 4-2B; 2011-2012). During the second growing season, in early spring and autumn, the CP concentration was similar (P>0.05) between the two and four week grazing frequency treatments, but in late spring and summer, the pasture treatments grazed every two weeks had a CP concentration that was respectively 13.5% and an 18.8% greater than those grazed every four weeks (Figure 4-2B; 2012-2013).

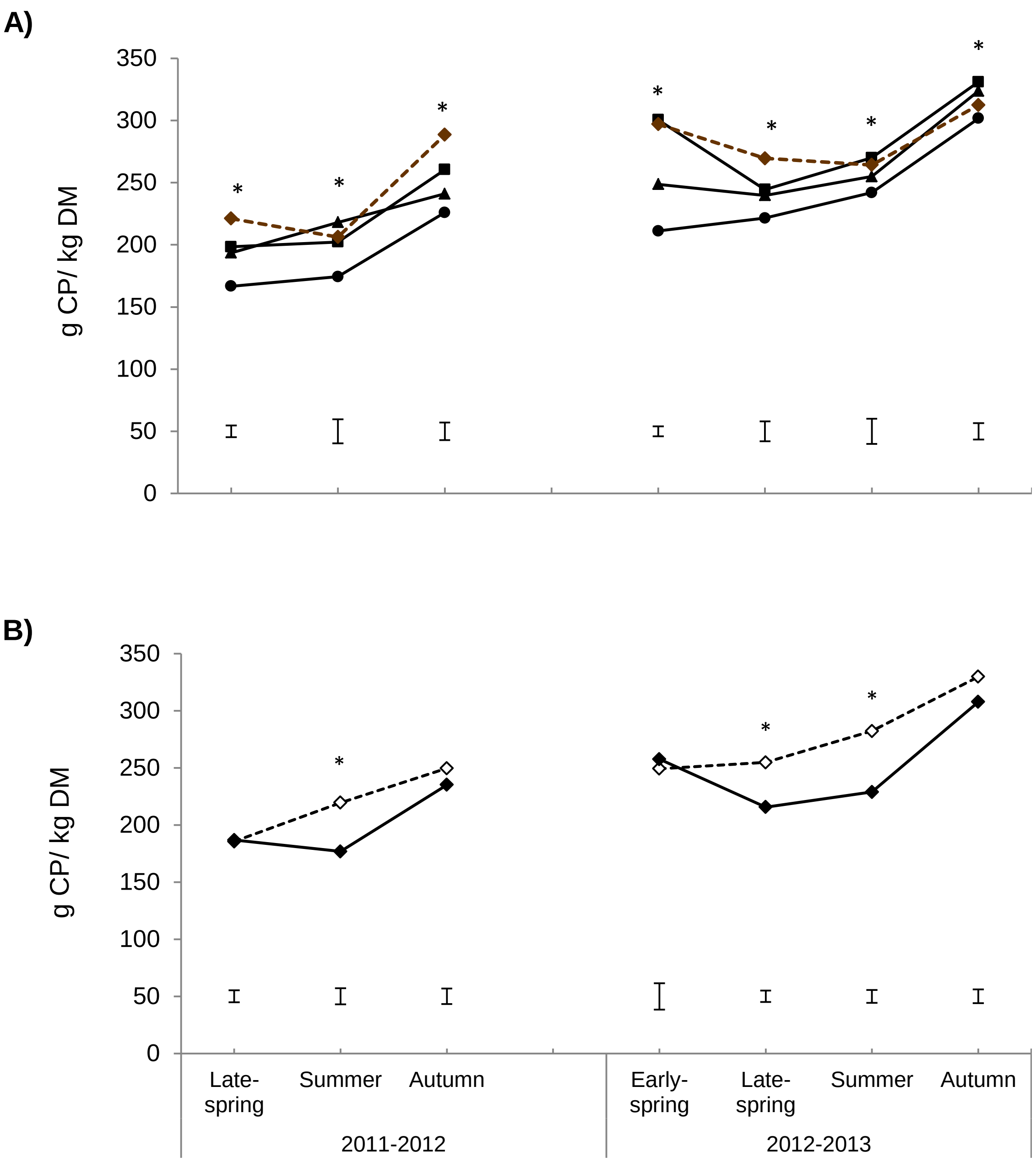

Figure 4-2 Seasonal concentration of crude protein (CP) in A) chicory (■), plantain (●), and the herb-clover mix (▲) pasture treatments and in the perennial ryegrass/ white clover pasture (♦ dashed lines) and B) in the pasture treatments by grazing every two (dashed line) or every four week (solid line) intervals during the first (2011-2012) and the second (2012-2013) growing season. Vertical bars represent the standard error of the mean (SEM). * indicate values that significantly differ (P<0.05) in each season.

4.4.4 Neutral detergent fibre and acid detergent fibre

The NDF and ADF concentrations were affected for an interaction (P<0.05) between pasture treatment and season in the first (2011-2012) and second (2012-2013) growing seasons. During the first growing season, in late spring and summer, the NDF (228 and 280 g NDF/ kg DM, respectively) and ADF concentrations (175 and 198 g ADF/kg DM, respectively) of plantain was greater (P<0.05) than the herb-clover mix which in turn had a greater (P<0.05) NDF (212 and 228 g NDF/kg DM, respectively) and ADF (150 and 165 g ADF/kg DM, respectively) concentrations than chicory (183 and 179 g NDF/kg DM, respectively and 141 and 137 g ADF/kg DM, respectively) (Figure 4-3; 2011-2012). In autumn, plantain and the herb-clover mix pasture had similar (P>0.05) NDF (198 g NDF/ kg DM) and ADF (154 g ADF/ kg DM) concentrations which were greater (P<0.05) than chicory (139 g NDF/kg DM and 106 g ADF/kg DM) (Figure 4-3; 2011-2012).

The NDF concentration of chicory did not differ (P>0.05) between late spring and summer, but the NDF concentration of plantain and the herb-clover mix increased (P<0.05) in summer when compared to late spring (Figure 4-3A; 2011-2012). The ADF concentration of chicory and the herb-clover mix remained similar (P>0.05) from late spring to summer, whilst the ADF concentration of plantain increased (P<0.05) in summer compared with late spring (Figure 4-3B; 2011-2012). In autumn, the NDF and ADF concentrations of chicory, plantain and the herb-clover mix declined and were lower (P<0.05) than in late spring and summer (Figure 4-3; 2011-2012).

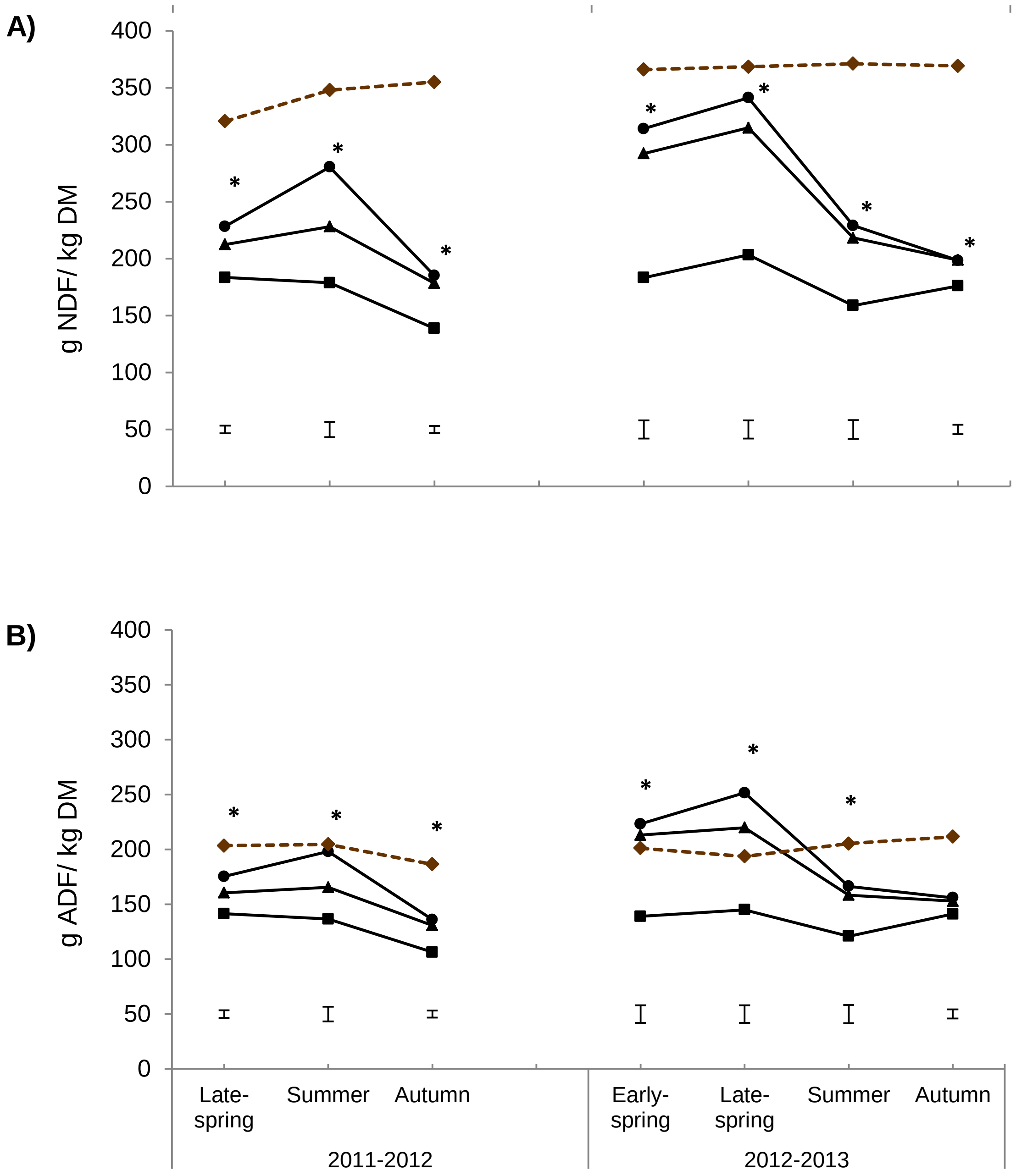

Figure 4-3 Seasonal concentration of A) neutral detergent fibre (NDF) and B) acid detergent fibre (ADF) in chicory (■), plantain (●), and herb-clover mix (▲) pasture treatments and in the perennial ryegrass/ white clover pasture (♦ dashed lines) during the first (2011-2012) and the second (2012-2013) growing seasons. Vertical bars represent the standard error of the mean (SEM). * indicate values that significantly differ (P<0.05) in each season.

During the second growing season, in early spring and summer, both plantain and the herb-clover mix had similar (P>0.05) concentrations of NDF (303 and 224 g NDF/ kg DM, respectively) and ADF (218 and 164 g ADF/ kg DM, respectively) that were greater (P<0.05) than chicory (183 and 159 g NDF/kg DM, respectively and 139 and 121 g ADF/kg DM, respectively) (Figure 4-3; 2012-2013). In late spring, the NDF (341 g NDF/kg DM) and ADF (252 g ADF/ kg DM) concentrations of plantain was greater (P<0.05) than that of the herb-clover mix (315 g NDF and 220 g ADF/ kg DM) and was lowest in chicory (203 g NDF and 150 g ADF/ kg DM) (Figure 4-3; 2012-2013). The NDF concentration in autumn was higher (P<0.05) for plantain and the herb clover-mix (198 g NDF/ kg DM) than for chicory (176 g NDF/ kg DM), while the ADF concentration was similar (P>0.05) in the three pastures (150 g ADF/ kg DM) during autumn (Figure 4-3; 2012-2013). The NDF concentration in plantain and the herb-clover mix increased (P<0.05) from early spring to late spring, then declined in summer and remained similar (P>0.05) in autumn (Figure 4-3; 2012-2013).

The NDF concentration of perennial ryegrass/white clover was numerically greater than the herbs and herb-clover mix in each season during the first (ranging from 320 to 355 g NDF/kg DM) and second (ranging from 366 to 371 g NDF/kg DM) growing seasons (Figure 4-3A). During the first growing season, the ADF concentration of perennial ryegrass/white clover was numerically higher in late spring (204 g ADF/kg DM), summer (205 g ADF/kg DM) and autumn (184 g ADF/kg DM compared to the three pasture treatments (Figure 4-3B; 2011-2012). However, during the second growing season, the ADF concentration of perennial ryegrass/white clover in early and late spring (201 and 194 g ADF/kg DM, respectively) was numerically lower than plantain and the herb-clover mix pastures which was numerically greater than chicory, but perennial ryegrass/white clover in summer and autumn (205 and 212 g ADF/kg DM, respectively) had a ADF concentration greater than chicory, plantain and the herb-clover mix (Figure 4-3B; 2012-2013).

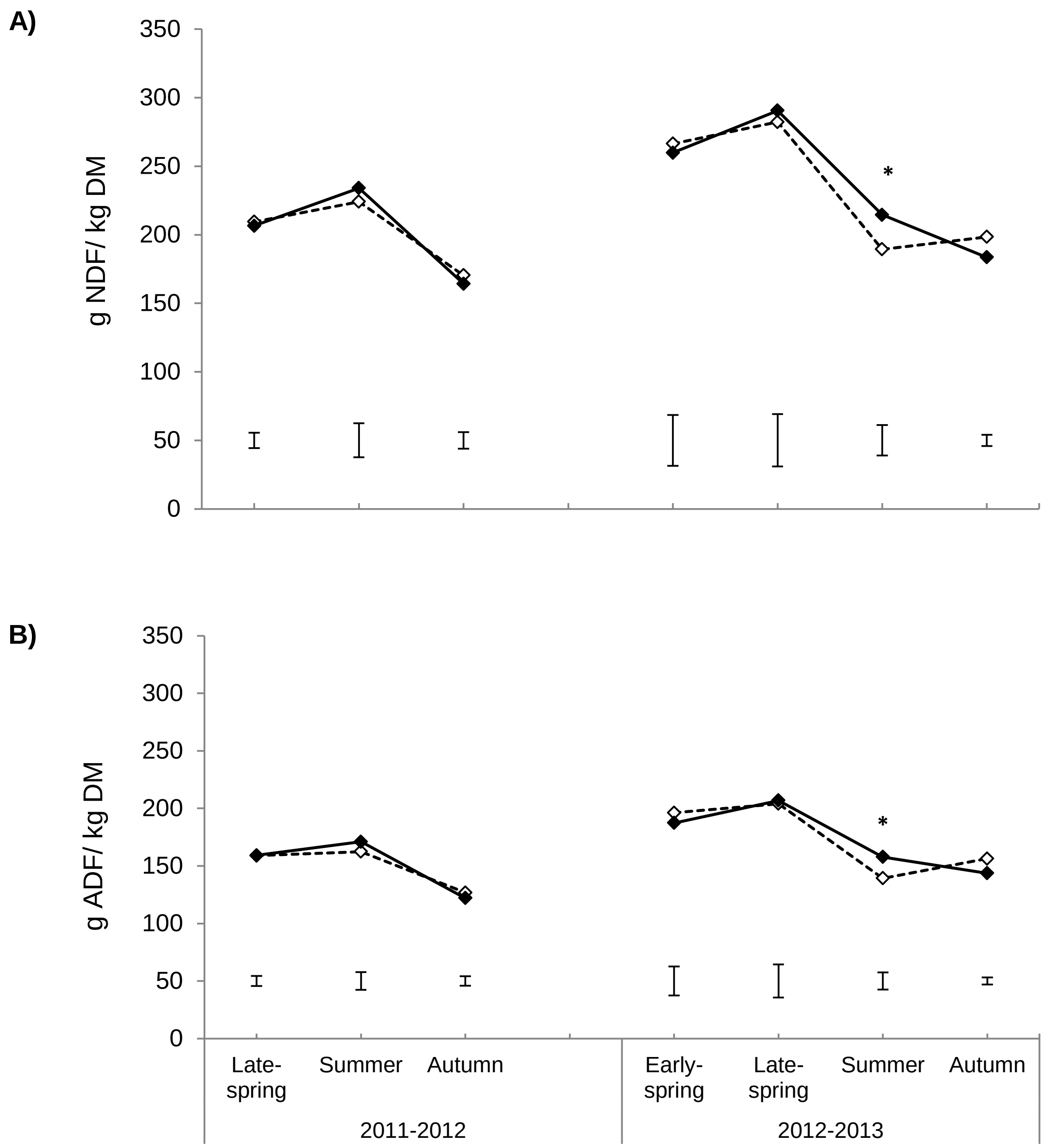

Figure 4-4 Seasonal concentration of A) neutral detergent fibre (NDF) and (B) acid detergent fibre (ADF) in the pasture treatments by grazing every two (dashed lines) or every four week (solid line) frequencies during the first (2011-2012) and second (2012-2013) growing seasons. Vertical bars represent the standard error of the mean (SEM). * Indicate values that significantly differ (P<0.05) in each season.

Grazing frequency did not affect (P>0.05) the NDF and ADF concentrations in the herbs and herb-clover mix in the first growing season (Figure 4-4; 2011-2012) and no interactions (P>0.05) were detected. However, in the second growing season, there was an interaction (P<0.05) between grazing frequency and season for NDF and ADF concentrations (Figure 4-4; 2012-2013). In summer, the NDF and ADF concentration in the herb and herb-clover mix pastures in the two weeks grazing frequency treatment were lower (P<0.05) than that of the four weeks grazing frequency treatment (Figure 4-4). However, in early spring, late spring, and autumn the NDF and ADF concentration were similar (P>0.05) between grazing frequency (Figure 4-4; 2012-2013).

4.4.4.1 Cellulose, hemicellulose, and lignin

The concentration of cellulose, hemicellulose and lignin in the pastures in the different seasons of each growing season is presented in Table 4-1. The concentration of cellulose and hemicelluloses was affected (P<0.05) by pasture and season in both growing season. During the first growing season, in late spring and autumn, the cellulose concentration was similar (P<0.05) between chicory, plantain and the herb-clover mix pastures (Table 4-1; 2011-2012). In summer, the cellulose concentration of plantain was greater (P<0.05) than that of the herb-clover mix, with chicory having the lowest (P<0.05) cellulose concentration (Table 4-1; 2011-2012). The hemicellulose concentration in late spring and autumn was greater (P<0.05) in plantain and the herb-clover mix pastures compared to chicory, and in summer, the concentration of hemicellulose was greater (P<0.05) in plantain than in the herb-clover mix and lowest (P<0.05) in chicory (Table 4-1; 2011-2012).

Table 4-1 Seasonal concentration (g/kg DM) of cellulose, hemicellulose, and lignin in chicory, plantain, and the herb-clover mix pastures and in perennial ryegrass/white clover and in the pasture treatments by grazing every two or four week frequencies during the first (2011-2012) and the second (2012-2013) growing seasons.

	2011-2012			2012-2013			
	Late spring	Summer	Autumn	Early spring	Late spring	Summer	Autumn
Cellulose							
Pasture							
Chicory	81.2 ^{ax}	82.4 ^{cx}	68.4 ^{ay}	61.8^{bx}	52.7^{bx}	69.0 ^{bxy}	72.6 ^{ay}
Plantain	79.3 ^{ax}	122.6 ^{ay}	76.5 ^{ax}	73.1^{ax}	77.7^{axy}	91.0 ^{axy}	80.2 ^{ay}
Herb-clover	89.6 ^{ax}	108.5 ^{by}	77.9 ^{az}	70.1^{ax}	88.5^{ax}	100.6 ^{ay}	79.6 ^{ax}
Ryegrass[1]	177.2	176.9	166.5	183.4	176.0	175.9	188.7
SEM[2]	3.13	5.23	2.71	3.40	6.15	3.34	4.69
Grazing frequency							
2 weeks	82.2	95.9 ^{b}	75.6	67.1	65.7	82.6	83.1
4 weeks	84.5	113.0 ^{a}	72.9	69.5	80.3	91.1	71.9
SEM[2]	2.79	5.93	2.43	3.36	6.55	4.79	4.17
Hemicellulose							
Pasture							
Chicory	42.1 ^{bx}	42.2 ^{cx}	32.3 ^{by}	44.2 ^{bxy}	58.3 ^{bx}	37.9 ^{bxy}	34.9 ^{ay}
Plantain	52.9 ^{ax}	82.3 ^{ay}	49.0 ^{ax}	90.7 ^{ax}	89.6 ^{ax}	62.5 ^{ay}	42.3 ^{ay}
Herb-clover	51.9 ^{ax}	62.7 ^{by}	47.5 ^{ax}	79.2 ^{ax}	95.0 ^{ax}	59.8 ^{ay}	45.8 ^{ay}
Ryegrass[1]	117.2	143.3	168.6	164.9	174.7	165.9	157.5
SEM[2]	2.04	2.89	1.32	4.02	7.14	3.55	1.16
Grazing frequency							
2 weeks	50.60	61.83	43.63	70.22	78.21	50.06	42.06
4 weeks	47.33	63.01	42.24	72.54	83.72	56.78	39.93
SEM[2]	2.05	5.00	2.29	7.24	8.09	4.72	1.68
Lignin							
Pasture							
Chicory	60.15 ^{bx}	54.16 ^{bx}	38.00 ^{by}	77.20^{bx}	92.19^{cx}	51.90 ^{by}	68.46 ^{ay}
Plantain	96.01 ^{ax}	75.43 ^{ay}	59.53 ^{az}	150.15^{ay}	173.89^{ax}	75.45 ^{az}	75.77 ^{az}
Herb-clover	70.89 ^{ax}	56.98 ^{by}	52.93 ^{ay}	142.86^{ax}	131.43^{bx}	57.73 ^{by}	73.32 ^{by}
Ryegrass[1]	26.24	27.63	19.94	17.70	17.71	29.38	22.92
SEM[2]	3.68	3.94	3.19	7.75	9.11	4.32	4.58
Grazing frequency							
2 weeks	76.68	66.42 ^{a}	51.22	129.02	138.40	56.74	73.28
4 weeks	74.68	57.96 ^{a}	49.08	117.79	126.60	66.65	71.74
SEM[2]	1.92	4.67	2.15	7.77	8.67	5.11	2.03

$^{a,\ b,\ c}$ superscripts indicate that values within columns are significantly different (P<0.05). $^{x,\ y,\ z}$ superscripts indicate that values within rows are significantly different (P<0.05)

[1] Perennial ryegrass pasture was not included in the statistical analysis; [2] SEM, standard error of the mean.

During the first growing season, the cellulose and hemicellulose concentration increased (P<0.05) from late spring to summer in plantain and the herb-clover mix, and then declined in autumn, with values similar (P>0.05) to late spring. Chicory had a cellulose concentration that was similar (P>0.05) in both late spring and summer but lower (P<0.05) in autumn (Table 4-1; 2011-2012). During the second growing season, in early spring, late spring, and summer both cellulose and hemicellulose concentration were greater (P<0.05) in plantain and the herb-clover mix pastures than in chicory (Table 4-1; 2012-2013). In autumn, the concentrations of cellulose and hemicellulose were similar (P>0.05) between the herbs and herb-clover mix pastures (Table 4-1; 2012-2013).

Grazing frequency did not affected (P<0.05) the concentration of hemicellulose in the herb and herb-clover mix pastures, but the concentration of cellulose was affected (P<0.05) for an interaction between grazing frequency and season only in the first growing season (Table 4-1; 2011-2012). In summer, the cellulose concentration in the pasture treatments grazed every four weeks was greater (P<0.05) than in the pasture treatments grazed every two weeks (Table 4-1; 2011-2012). However, in late spring and autumn, the cellulose concentration was similar (P>0.05) between both grazing frequency (Table 4-1; 2011-2012). During the second growing season, the cellulose and hemicellulose concentration in the herb and herb-clover mix pastures was not affected (P>0.05) by grazing frequency nor were there any interactions (P>0.05) detected (Table 4-1; 2011-2012).

Lignin content was typically greater (P<0.05) in plantain compared to chicory (Table 4-1). In the first growing season, chicory and the herb-clover mix had similar (P>0.05) lignin content in summer which was lower (P<0.05) than plantain (Table 4-1; 2011-2012). In late spring and autumn, plantain and the herb-clover mix had a similar (P>0.05) content of lignin which was higher (P<0.05) than chicory (Table 4-1; 2011-2012). During the second growing season, in early and late spring, the lignin content of chicory was lower (P<0.05) than plantain and the herb clover mix, but in summer and autumn, lignin content was similar between the herb pastures (Table 4-1; 2012-2013). Grazing frequency did not affect (P>0.05) lignin content and no interactions (P<0.05) were detected in either the first or the second growing season (Table 4-1).

4.4.5 Hot water soluble carbohydrates

During the first growing season, the HWSC concentration of chicory in late spring (182 g HWSC/kg DM) and autumn (138 g HWSC/kg DM) was greater (P<0.05) than plantain and the herb-clover mix (166 and 112 g HWSC/kg DM in late spring and autumn, respectively) (Figure 4-5A; 2011-2012). In summer, chicory and plantain had similar (P>0.05) HWSC concentrations (142 g HWSC/kg DM) which were greater (P<0.05) than that of the herb-clover mix (124 g HWSC/kg DM). The HWSC concentration of perennial ryegrass/white clover pasture in late spring, summer, and autumn (109, 118, and 94 g HWSC/kg DM, respectively) was numerically lower than the chicory, plantain, and herb-clover mix pastures (Figure 4-5A; 2011-2012).

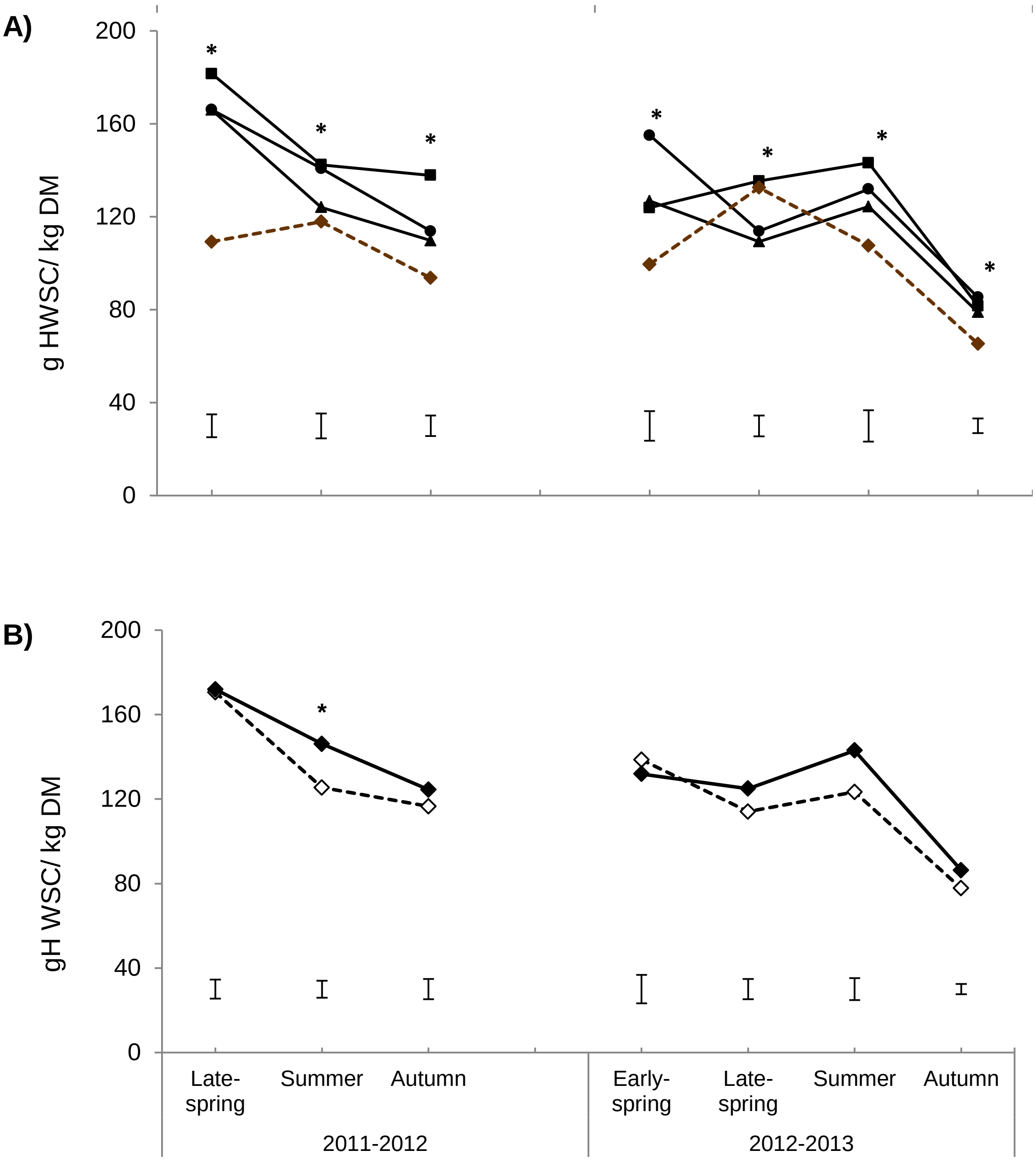

Figure 4-5 Seasonal concentration of hot water soluble carbohydrates (HWSC) in: A) chicory (■), plantain (●), and the herb-clover mix (▲) pasture treatments and in perennial ryegrass/white clover (♦ dashed lines) and in B) the pasture treatments by grazing every two (dashed lines) or every four week (solid lines) frequencies during the first (2011-2012) and the second (2012-2013) growing season. Vertical bars represent the standard error of the mean (SEM). * indicate values that significantly differ (P<0.05) in each season.

During the second growing season, in early spring, plantain had a HWSC concentration (155 g HWSC/kg DM) greater ($P<0.05$) than chicory and the herb-clover mix (125 g HWSC/kg DM) (Figure 4-5A; 2012-2013). In late spring and summer, HWSC concentration was greater ($P<0.05$) in chicory (135 and 143 g HWSC/kg DM, respectively) than either plantain or the herb-clover mix (112 and 128 g HWSC/kg DM in late spring and summer, respectively) (Figure 4-5A; 2012-2013). In autumn, the herb and herb-clover mix pastures had similar ($P>0.05$) HWSC concentrations (82 g HWSC/kg DM) (Figure 4-5A; 2012-2013).

Perennial ryegrass/white clover pasture in early spring, summer, and autumn had a HWSC concentration (100, 108, and 65 g HWSC/kg DM, respectively) numerically lower than the three pasture treatments, but in late spring, the HWSC of perennial ryegrass/white clover (133 g HWSC/kg DM) was similar to chicory and higher than plantain and the herb-clover mix (Figure 4-5A; 2012-2013).

Grazing frequency affected ($P<0.05$) the HWSC concentration in the first growing season (Figure 4-5B; 2011-2012). In late spring and autumn, the HWSC concentration was similar ($P>0.05$) between both grazing frequencies, but in summer, the pasture treatments grazed every four weeks had greater ($P<0.05$) HWSC concentration than those grazed every two weeks. During the second growing season, there was not effect ($P>0.05$) of grazing frequency on the HWSC concentration of the pastures treatments between seasons (Figure 4-5B; 2012-2013).

4.4.7 Ash

The ash content of chicory, plantain, and the herb-clover mix pastures was similar (P>0.05) at the beginning of the first (late spring; 131 g ash/kg DM) and second (early spring; 118 g ash/kg DM) growing seasons (Figure 4-6A). However, in summer (148 g ash/kg DM) of the first growing season, and in late spring and summer (122 and 131 g ash/kg DM, respectively) of the second growing season chicory had greater (P<0.05) ash content than plantain and the herb-clover mix in both the first (136 g ash/kg DM in summer) and second growing seasons (111 and 122 g ash/kg DM in late spring and summer, respectively) (Figure 4-6A). In autumn, the ash content of plantain was higher (P<0.05) in the first (154 g ash/kg DM) and second (155 g ash/kg DM) growing seasons compared to chicory and the herb-clover mix (148 and 146 g ash/kg DM, respectively) (Figure 4-6A; 2011-2012). Perennial ryegrass/white clover showed an ash content lower than the pasture treatments in the first (ranging from 118 to 123 g ash/kg DM) and second (ranging from 94 to 112 g ash/kg DM) growing seasons (Figure 4-6A).

Grazing frequency affected (P<0.05) the ash content in both growing seasons (Figure 4-6B). During the first growing season, in late spring and autumn, the ash content was not affected (P>0.05) by grazing frequency; however, in summer, the pastures grazed every two weeks had greater (P<0.05) ash content than those grazed every four weeks (Figure 4-6B; 2011-2012). During the second growing season, in early spring there was no effect (P>0.05) of grazing frequency; however, in late spring, summer and autumn, the pastures grazed every two weeks had higher (P<0.05) ash content than those grazed every four weeks (Figure 4-6B; 2012-2013).

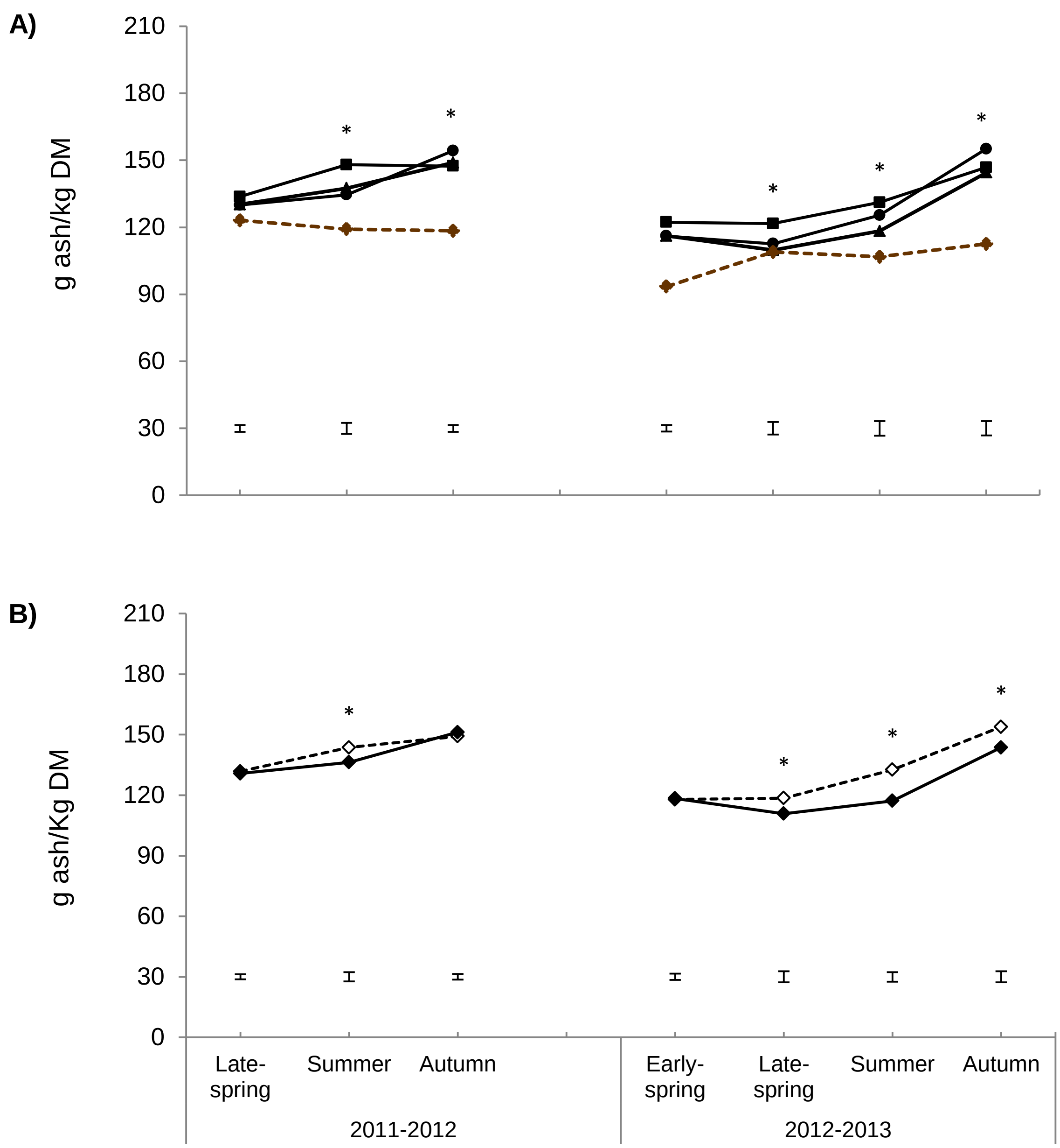

Figure 4-6 Seasonal ash content of: A) chicory (■), plantain (●), and the herb-clover mix (▲) pasture treatments and in perennial ryegrass/white clover (♦ dashed lines) pasture and in B) in the pasture treatments by grazing every two (dashed lines) or every four week (solid lines) frequencies during the first (2011-2012) and the second (2012-2013) growing season. Vertical bars represent the standard error of the mean (SEM). * indicate values that significantly differ (P<0.05) in each season.

4.5 DISCUSSION

Chicory, plantain, and the herb-clover mix pastures can be considered of high quality for lactating dairy cows, due to their ME being above 11 MJ ME/ kg DM, a NDF concentration not exceeding the 350 g NDF/kg DM (Hodgson and Brookes, 1999) and a CP concentration suitable for milk production (160-180 g CP/kg DM; NRC, 2001). The low ADF concentration typically observed in these three pastures suggests that other sources of forage would be needed in order to provide the minimum 210 g ADF/kg DM in rations for dairy cows to support a good rumen health and avoid depressions in milk fat production (NRC, 2001). In New Zealand, herb and herb-clover-mix pastures are generally provided as supplementary crops during summer and autumn to alleviate the limitations of a diet solely based on perennial ryegrass (Minnee et al., 2012). If utilized in this manner, nutritional imbalances are unlikely to occur. However, perennial ryegrass/white clover pasture in this study showed an ADF concentration below the minimum recommended and extra care should be considered. Additionally, the presence of secondary compounds in plantain, catalpol, aucubin, and acteoside has created interest in the potential for positive effects on animal health (Kemp et al., 2014; Stewart, 1996; Tamura and Nishibe, 2002) but their impact on rumen fermentation is unclear and will be investigated further in Chapter 6.

Chicory typically exhibited a higher nutritive value than plantain, with greater CP, ME, HWSC and less fibre (Fraser and Rowarth, 1996; Gregorini et al., 2013; Labreveux et al., 2006; Lee et al., 2015). The CP concentration of chicory and plantain found in the present study (Figure 4-2A) was similar to values previously reported (Brown and Moot, 2004; Cave et al., 2015; Labreveux et al., 2006; Lee et al., 2015; Volesky, 1996). The CP concentration in legumes, such as lucerne (*Medicago sativa* L.) and red clover, is high compared to both herbs (Barry, 1998; Brown and Moot, 2004; Stewart, 1996), and is positively related with legume content in a mixed sward (Deak et al., 2009; Deak et al., 2007). This resulted that the herb-clover mix pasture, containing chicory, plantain, red and white clover, had a CP concentration similar to chicory but higher than that of solely plantain. The NDF concentrations obtained in chicory and plantain in the current study (Figure 4-3A) were lower than values previously reported (Labreveux et al., 2006; Lee et al., 2015; Sanderson et al., 2003), which was also evident in the herb-clover mix. The three pastures had a NDF concentration consistently lower than perennial ryegrass (Barry, 1998). The cellulose and hemicellulose concentrations were lower, but lignin was higher in both chicory and plantain compared to perennial ryegrass as reported previously (Barry, 1998; Swainson and Hoskin, 2006) and consequently also in the herb-clover mix pasture.

Chicory showed a higher ME content that was more stable through the seasons than plantain and the herb-clover mix (Barry, 1998). Greater levels of pectin have been reported for chicory (Barry, 1998; Jackson et al., 1996; Sun et al., 2011; Sun et al., 2012). Thus, the ME content in chicory may reflect greater accumulation of pectin, although pectin levels were not measured in this study. The lower ME content in plantain and the herb-clover mix pastures is likely associated with their greater concentration of structural components (NDF and ADF) and lignin compared to chicory (Tessema et al., 2010). Additionally, chicory had consistently lower concentrations of cellulose and hemicellulose (SC), and lower concentrations of lignin compared to plantain and the herb-clover mix. This suggests that the ratio of RFC to SC is likely to be higher in chicory than plantain and the herb-clover mix pastures. A greater ratio RFC to SC contributes to the higher DM degradability of chicory compared to plantain and herb-clover mix as reported in previous studies (Barry, 1998; Burke et al., 2000). This may allow for greater DMI and hence increased animal production. However, herbage DMI also depends on feed acceptance by animals, and herbage intake can be affected by both physical and chemical traits of the herbage (Ungar and Noy-Meir, 1988). Both herbs have been reported as palatable to ruminants (Stewart, 1996; Barry, 1998), but reproductive stems in plantain have been reported to have palatability problems during late summer and autumn in lambs (Fraser and Rowarth, 1996; Swainson and Hoskin, 2006). In this sense, the diet selection of dairy cows for chicory and plantain as pure swards and in a herb-clover mix pasture will be evaluated in chapter 5.

The seasonal changes observed in CP and NDF concentrations follow a similar pattern in all three of the pasture types and perennial ryegrass/white clover. The low CP and high NDF concentrations observed in late spring are likely due to herbage maturity (Fulkerson et al., 1998). The high CP and low NDF concentration observed in autumn are likely due to an increased proportion of leaf in the forage (Minson, 2012), as leaves are higher in NDF and lower in CP than reproductive stems (Lee et al., 2015). Moreover, the decline in NDF from late spring to summer in plantain and the herb-clover mix led to an increase in the ME content in these pastures in comparison to chicory and perennial ryegrass. Although the ME content in perennial ryegrass was moderate (averaging 10.5 MJ ME/ kg DM; Minnee et al., 2012), in summer (February) the herbs and herb-clover mix pastures had consistently greater ME content than perennial ryegrass. These findings suggest that chicory, plantain, and the herb-clover mix may be useful for improving animal performance during the time of year when the nutritive value of ryegrass is typically limited (Chapman et al., 2008; Kemp et al., 2010).

During late spring and summer in the first year, plantain had the lowest CP concentration (< 200 g CP/kg DM) compared to chicory and the herb-clover mix; however, this was still adequate for a cow producing 15 L milk/d (Waugh et al., 1998). Previous works have also reported low CP concentrations of plantain during spring (107 – 120 g CP/kg DM; (Hoskin et al., 2006; Swainson and Hoskin, 2006) and summer (160 g CP/kg DM; (Cave et al., 2015). This is likely due to the higher stem production in plantain compared with chicory (Lee et al., 2015; Sanderson et al., 2003) and the lower CP concentration in stems compared with leaves (Fraser and Rowarth, 1996; Lee et al., 2015). Similarly, Lee et al. (2015) reported a lower CP concentration in plantain during spring and summer associated with a greater stem production (42 - 48%) compared to chicory (12 - 33%). It has also been reported that soil moisture stress causes a greater reduction of CP in plantain than has been observed for chicory or red clover (Kemp et al., 2010). Sowing plantain in combination with legume species such as red and white clover is recommended as a means to provide N fixation and to improve the overall nutritive value of the mixed sward (Kemp et al., 2014). Consequently, the herb-clover mix was similar to plantain in regards to its ME and fibre concentrations, but had a higher CP concentration than the pure plantain.

Grazing frequency affected herbage nutritive value, whereby grazing the herb and herb-clover mix pastures every two weeks improved CP and reduced NDF concentrations compared to grazing every four weeks. Several studies have consistently reported a greater CP concentration when chicory and plantain were defoliated at shorter rather than longer intervals (Holden et al., 2000; Labreveux et al., 2006; Lee et al., 2015; Volesky, 1996). This has been associated with a decline in the quality of the leaves; as they age and at higher herbage masses, the CP concentration of the herbage is expected to be lower than at lower herbage masses (Lee et al., 2015). Nevertheless, the effect of defoliation intervals on NDF concentration has been varied when defoliation has been under mowing or grazing conditions. Both chicory and plantain defoliated every three weeks had lower NDF concentration compared to those defoliated every five weeks (Sanderson et al., 2003). Recently, Lee et al. (2015) reported that during the first growing season, the defoliation of chicory from 15 to 45 cm of extended leaf height (ELH) did not affect the NDF concentration, but for plantain defoliating at greater ELH (from 15 to 55 cm), there was an increase in the NDF concentration. However, both Holden et al. (2000) and Labreveux et al. (2006) reported no effect of grazing frequency on NDF concentration either in chicory or plantain when grazed by cattle. These differences in nutritive value may result from a decreased development of stems when grazing every two weeks than grazing every four weeks, causing a higher leaf to stem ratio, although this was not confirmed in this study.

4.6 CONCLUSIONS

The herbage produce for chicory and plantain, in pure swards or the herb-clover mix was of high quality for lactating dairy cows throughout two consecutive growing seasons. During late spring and summer frequent grazing (two week intervals) improved the pasture quality, by increasing CP and decreasing fibre concentration. This suggests that the lower CP concentration in plantain and higher fibre concentration in the herb-clover mix observed during late spring and summer could be improved by grazing every two weeks. In contrast, grazing chicory at frequent intervals is not recommended, nor is it likely necessary as the overall nutritive value of chicory, irrespective of grazing frequency, was consistently greater than that of plantain.

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5 SELECTIVE GRAZING OF CHICORY AND PLANTAIN IN PURE SWARDS OR IN MIXED SWARDS BY LACTATING DAIRY COWS

5.1 ABSTRACT

Ruminants can selectively graze one species over others in mixed swards which may influence their herbage intake. This research reports two studies that had the overall objective of examining the diet selection and grazing preference of dairy cows for chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.), as pure swards and in a herb-clover mix, which had been managed under two grazing frequencies (two and four week intervals). Study One examined the diet selection of dairy cows for chicory, plantain, red clover (*Trifolium pratense* L.), and white clover (*T. repens* L.) sown in a herb-clover mix during late spring, summer and autumn using transects. Ten transects (2 m long each) were randomly placed in each herb-clover mix plot (n=10) and individual chicory, plantain, red, and white clover plants were tagged according their proportion in the swards in each season. Study Two examined the grazing preference of dairy cows on adjacent pastures of chicory, plantain, and an herb-clover mix during summer, early autumn and late autumn by direct behavioural observation and also by using global positioning system (GPS) collars on each cow to track their positions. Dairy cows preferred the herb-clover mix over chicory or plantain, from the swards grazed every four weeks. Dairy cows, grazing a herb-clover mix, indiscriminately selected chicory, plantain and red clover irrespective of their proportion in grazed horizon (>7 cm) and avoided white clover due to its greater proportion in the lower strata (<7 cm) of the swards. The diet selection and grazing preference of dairy cows for chicory and plantain varied among seasons. Chicory had a consistent high selection index and cows spent a greater proportion of time grazing the pure chicory sward. However, plantain had an increase selection index and proportion of time grazing the pure plantain sward which was likely due to a lower stem production.

Keywords: Chicory, plantain, herb-clover mix, diet selection, preference, dairy cows

5.2 INTRODUCTION

Diet selection of grazing animals affects their herbage intake on pasture. Dry matter intake (DMI) of high quality herbage is a key factor to improve milk production of dairy cows. However, under grazing conditions, sward characteristics such as, species availability (herbage mass) and accessibility (vertical distribution), and plant palatability affect the diet selection of animals, and as result, their herbage intake (Baumont et al., 2000; Dumont et al., 2002; Ungar and Noy-Meir, 1988; Waghorn and Clark, 2004). Knowing the diet selection and grazing preferences of dairy cows for herb pastures may assist farmers to increase DMI and improve animal production (Pain et al., 2010).

Chicory (*Cichorium intybus* L.), plantain (*Plantago lanceolata* L.) and herb-clover mix pastures produce herbage of high quality. Both chicory and plantain are rapidly degraded in the rumen than perennial ryegrass thus resulting in a faster rumen clearance (Burke et al., 2000; Hoskin et al., 1995; Kusmartono et al., 1997), allowing for higher DMI in animals grazing these pastures. Both herbs have been reported to be palatable to sheep and cattle (Barry, 1998; Stewart, 1996). Furthermore, when given the choice, in indoor trials, weaned lambs had similar preference for chicory and plantain in spring and summer, but higher preferences were observed for red clover (*T. pratense* L.) in both seasons (Pain et al., 2014; Pain et al., 2010). This suggested that a high preference for chicory and plantain might be obtain by dairy cows grazing these herb pastures.

Research, under grazing conditions, on the diet selection and preference of chicory and plantain has been varied between seasons. When adjacent monocultures of each species were offered, in an outdoor trial, the grazing preference of sheep for chicory was greater than plantain in early autumn, but similar in late autumn (Pain et al., 2014). Recently, ewes grazing mixed swards containing chicory, plantain, red and white clover showed a similar selection for chicory and plantain from early spring to autumn (Cave et al., 2015); however, ewes have also been shown to have a greater selection for chicory than plantain in late summer (Pain et al., 2014). Previous work reported that, when offered to lambs, poor palatability of plantain was associated with higher stem development of the plant during late summer and autumn compared to chicory and red clover (Fraser and Rowarth, 1996; Swainson and Hoskin, 2006). The lower selection of plantain compared to chicory when grazed by lambs in different seasons is likely related to stem development in plantain reducing its palatability.

Previous studies have used ewes and lambs to investigate the diet selection and grazing preferences of herbs and legumes and the impact of grazing management (Cave et al., 2015; Pain et al., 2014; Pain et al., 2010). There is limited information on the diet selection and grazing preference of dairy cows for chicory and plantain, as pure swards and herb-clover mix pastures. The objective of this study was to determine the effect of the swards structure on the diet selection for chicory, plantain, red and white clover within a herb-clover mix pasture and the grazing preference for chicory, plantain, and herb-clover mix pastures when managed with a grazing frequency of either two or four weeks over different seasons.

5.3 MATERIAL AND METHODS

The research reported here comprised two studies, both conducted over three seasons (late spring, summer, and autumn) during 2012 and 2013.

- Study One: Seasonal diet selection of dairy cows grazing within a herb-clover mix pasture, determined using plant transects.

- Study Two: Seasonal diet selection of dairy cows grazing pure swards of chicory and plantain, and a herb-clover mix pasture, determined by grazing behavioural observations.

5.3.1 Study One: Diet selection of dairy cows grazing a herb-clover mix pasture

Diet selection of dairy cows grazing established herb-clover mix pasture (two years old), containing chicory, plantain, red and white clover was evaluated over three different seasons: late spring (December, 2012); summer (February, 2013); and autumn (April, 2013). Ten plots (300 m^2 each) of the herb-clover mix established in October, 2011 were sown as: plantain (cv.' Ceres Tonic'; 6 kg/ha), chicory (cv. 'Choice'; 6 kg/ha), red clover (cv. 'Sensation'; 6 kg/ha), and white clover (cv. 'Emerald' and 'Bounty'; 4 kg/ha). During the first growing season (2011-2012), these swards were grazed rotationally by lactating dairy cows at two different frequencies: every two or four weeks.

5.3.1.1 Plant measurements using transects

Ten transects (each 2 m long) were randomly placed in each plot. In each transect ten plants were tagged leaving a 20 cm space between each plant tagged. For each species the number of plants that were tagged was related to its relative abundance (RA) in the sward during each season (Table 5-1). To establish the RA of each species, the botanical composition of the herb-clover mix was measured prior to placing the transects.

Two 50 x 10 cm strips were randomly selected from each plot and cut to ground level with an electric shearing hand-piece. The samples were manually separated into species (plantain, chicory, red clover, and white clover), weeds (unsown species), and dead material, and then oven dried at 70 °C (approximately 48 h). The proportion of each species in the total dry matter (DM) was then calculated as the RA of each species (Table 5-1).

Individual chicory, plantain, red clover and white clover plants were tagged at the base of the plant close to the ground using coloured plastic wire. This resulted in 10 plants labelled per transect, 100 plants per plot, 500 plants per grazing frequency and 1000 plants in total in each season.

Table 5-1 Proportion (%), defined as the relative abundance of plantain, chicory, red and white clover during late spring, summer, and autumn in the herb-clover mix swards

Species	Late spring	Summer	Autumn
Plantain	56	34	34
Chicory	10	6	16
Red clover	9	12	15
White clover	26	37	35

The plots were grazed by lactating dairy cows immediately after the morning milking until the sward achieved a residual height of 70 - 100 mm (approximately 5 h). The cows were then removed from the plots and the tagged plants in each transect were checked and recorded. Whether or not a plant or stolon had been eaten was recorded as grazed or not grazed, respectively. A plant was considered to have been grazed if one or more leaves had been removed.

The selection index (Si), defined as the ratio of the RA of species *y* in the diet to that in the environment (Jacobs, 1974), was calculated for each species (based on the number of plants tagged per plot) as an indicator of animal selection using the following equation:

$$\mathrm{Si}_y = (C_y/D)/\mathrm{RA}_y$$

Where:

Si_y is the selection index calculated for species *y*

C_y is the number of times that species *y* was grazed

D_y is the sum of all the species were grazed

RA_y is the relative abundance of the species *y*

The selection index ranges from 0 to 1 for negative selection indicating avoidance or inaccessibility of the species, and from 1 to infinite indicating positive selection.

5.3.1.2 Sward measurements

Herbage mass in each plot was measured the day before grazing by cutting two randomly chosen 0.1 m^2 quadrats to ground level using an electric shearing hand-piece. The herbage samples were then washed and oven dried at 70 °C for approximately 48 h.

Botanical composition was assessed by taken three 0.04 m^2 quadrats from each plot. The botanical samples were cut at three different height strata: lower strata (0-7 cm); medium strata (7-20 cm); and upper strata (>20 cm) using a blade shearing. The samples within each height strata were manually separated into the following categories: chicory leaves; chicory stem; plantain leaves; plantain stem; red clover; white clover; weeds (including other species and grasses); and dead material. Each category was individually oven dried at 70 °C (approximately 48 h) to calculate their proportion of the total DM within each height strata and in the total sample.

5.3.2 Study Two: Preference of dairy cows for chicory, plantain or herb-clover mix pastures determined by grazing time

Grazing preference of dairy cows was assessed in pure chicory and pure plantain swards, and a herb-clover mix pasture in three different seasons: summer (8 and 9 January, 2013); early autumn (5 and 6 March, 2013); and late autumn (30 April and 1 May, 2013). All pastures had a previous grazing management of two grazing frequencies: every two or every four weeks.

The experiment was carried out in a randomised complete block design with a factorial arrangement of the pasture treatments (three pasture and two grazing frequencies) in four replicates (blocks). Each block (I - IV) represented four individually fenced trial paddocks containing the three pasture treatments (chicory, plantain, herb-clover mix) grazed at either two or four week intervals (Figure 5-1). The grazing area (69 x 26 m) for each trial paddock contained six equal areas of 300 m^2 for each pasture frequency treatment.

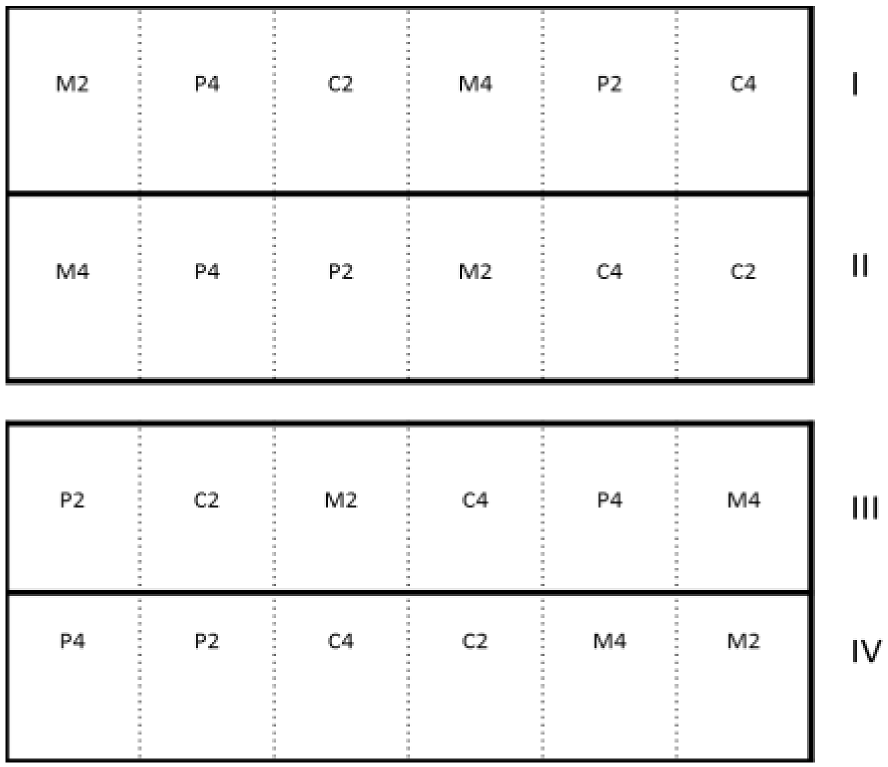

Figure 5-1 Distribution of the pasture treatments within block (I – IV). C2 = chicory grazed every two weeks, C4 = chicory grazed every four weeks, P2 = plantain grazed every two weeks, P4 = plantain grazed every four weeks, M2 = herb-clover mix grazed every two weeks, and M4 = herb-clover mix grazed every four weeks.

5.3.2.1 Animal behavioural observations

Ten lactating dairy cows from the No1 Dairy Farm herd at Massey University that had calved in spring were selected to be used in this study. All cows had grazed the chicory, plantain, and herb-clover mix pastures during the five months prior to this study and therefore had previous experience with the plot block layout and the pasture species to be tested. The day before the experiment the ten cows were separated from the dairy herd and painted on both sides with an individual number (1 - 10) using fluorescent stock paint to make it easier for the observers to recognise the animal.

Grazing behaviour was evaluated in the morning (AM) and afternoon (PM) for two consecutive days in the three seasons. The cows were milked twice a day (at 0630 and 1400 h) and immediately after milking the cows were placed in a trial paddock (block I –I V) to graze the pasture treatments during 2 h (Figure 5-1). Each paddock trial was grazed once, with the cows having access to all pasture treatments within each block. The cows grazed block I after the AM milking and block II after the PM milking on day one, and block III and IV after the AM and PM milking, respectively on day two.

The behavioural observations were recorded for 2 h in the AM and PM grazing. Two observers recorded the activity of each cow at 2 minute intervals, as eating (E), lying down (L), ruminating (R), walking (W), drinking water (D), or idling (I). The location of the cow on the pasture treatment was also recorded at the same 2 minute intervals. Once the cows had grazed for the 2 h, the animals were removed from the trial paddock and returned to graze a perennial ryegrass pasture. Cows were not fed supplements and had *ad-libitum* access to water.

5.3.2.2 Animal global positioning system

Global positioning system (GPS) was used to track cow position in the paddock during the period that behavioural observations were carried out. Each cow was fitted with a GPS collar to track their position (Figure 5-2). The GPS units were custom-made using Trimble® Lassen GPS modules programmed to allow continual tracking of satellites, and were powered by one 3.6-V, 19-Ah Tadiran battery to run continuously for seven days. The GPS unit was housed in a plastic container and mounted to an adjustable leather collar with an antenna (Trimble® Active). The collar was placed around the neck of the cow in such a way that the antenna was situated at the nape of the neck and the GPS unit was under the neck of the cow (Figure 5-2).

The GPS collars were programmed to register the cow's position whenever a cow moved ≥4 m or every 60 seconds if the cow did not move during that time. The GPS fix rate recording was set at 60 seconds intervals to improve the accuracy of recording animal position in the small plot area (300 m^2) of the pasture treatments evaluated. However, tree cover can cause errors on the accuracy of GPS recording (Rempel and Rodgers, 1997).

The GPS unit recorded date and time (GMT), latitude, longitude, and the number of satellites used. Once the behaviour observations in each season were completed, the GPS collars were retrieved from each cow. All records from the GPS units were downloaded to a computer and stored electronically.

Figure 5-2 A dairy cow fitted with a GPS collar

5.3.2.3 Sward measurements

Herbage mass and botanical composition of the pasture treatments during each period were determined by cutting three samples to ground level from each plot with an electric shearing hand-piece. The herbage mass samples from all plots were taken using a 0.1 m^2 quadrat and once cut, all samples were washed and oven dried at 70 °C (approximately 48 h). Botanical samples were taken by cutting a strip (50 x 10 cm) beside each herbage mass quadrat. The botanical composition samples were manually separated into the following categories: sown species; unsown species; and dead material, and were then oven dried individually at 70 °C (approximately 48 h). The proportion for each category species was calculated relative to the total dry weight.

5.3.3 Calculations and statistical analysis

All statistical analyses were conducted using the MIXED procedure in SAS (Version 9.3, SAS Institute, 2009). Means were compared using the least square means test and significance was declared at $P<0.05$.

5.3.3.1 Study One

The selection index was analysed using a three way ANOVA with species (chicory, plantain, red and white clover), grazing frequency (two or four week intervals) and season (late spring, summer, and autumn) as fixed effects and plot treated as a random effect to take account for the repeated measures through seasons .

The herbage mass in the herb-clover mix pasture was analysed using a two way ANOVA with grazing frequency and season included as fixed effects and the random effect of plot. The botanical composition, total and by strata, was analysed using a three way ANOVA. The total botanical composition included the species (chicory, plantain, red clover, white clover, weeds, dead material), grazing frequency (two and four weeks), and season (late spring, summer, autumn) as fixed effects. The proportion of plantain (leaves and stem), chicory (leaves and stem), red clover, white clover and weeds (other species and grasses) were analysed with sward strata (<7; 7-20; and >20 cm), grazing frequency (two and four week intervals), and season (late spring, summer, and autumn) included as fixed effects.

5.3.3.2 Study Two.

Grazing was calculated as the proportion of the time spent eating and preference was calculated as the proportion of time spent grazing in each pasture treatment for each cow, during the 2 h behaviour observation periods, in each trial paddock (I – IV). However, for the ten cows used in this study only eight cows were used in every season. Therefore, all data was calculated and analysed based on the records for those eight cows.

Repeated measures analysis was performed for the time spent grazing and the grazing preference, considering the individual cow as subject. The analyses of time spent grazing included the fixed effect of time of grazing (AM/PM) and season (summer, early autumn, late autumn), as well the interaction between grazing time and season. The grazing preference of dairy cows was analysed using the fixed effects of pasture treatments (chicory, plantain, herb-clover mix), grazing frequency (two and four weeks), time of grazing (AM/PM), and season (summer, early autumn, late autumn) and their interaction.

Herbage mass and the botanical composition of the chicory, plantain, and herb-clover mix pastures was analysed using a three way ANOVA with pasture treatments (chicory, plantain, herb-clover mix), grazing frequency (two and four weeks), and season (month) as fixed effects as well as their interaction. Block was treated as a random effect to take account for the repeated measures through seasons.

5.3.3.2.1 Interpretation of GPS records

GPS data recorded during the 2 h grazing period was entered to ArcGIS version 10.1 (ESRI, Redlands, California, USA) to investigate the relationship between cow position and grazing preference for the pasture treatments using geographic information system (GIS). Latitude and longitude data were used to generate a map layer of positions for cows in each observation period.

Cow's density was estimated using ArcGIS software. Cow density (intensity) defined as the density of points (number of points per unit area) was used to analyse the point pattern (Baddeley, 2008). This intensity (point density) may be constant (homogenous) or vary from location to location (heterogeneous). When the intensity is homogenous, it means that within a two-dimensional space, the point's distribution is uniform throughout the total area. However, intensity usually varies from place to place within a given area (heterogeneous). If the intensity is suspected to be heterogeneous, this can be estimated non-parametrically by quadrant counting and kernel density estimation.

The cows' density (intensity) in each plot area (pasture treatments) was estimated in each trial paddock (I - IV) during the AM (I and III) and PM (II and IV) grazing times for each season, using the kernel density estimation, also referred to as kernel smoothing (KS). The number of points in each plot area was calculated to estimate the mean duration that each cow spent in each pasture treatment.

Kernel smoothing is a non-parametric way of estimating the probability density function of a random variable. If x_1, x_2, ...,x_n ~ f is an independent and identically-distributed sample of a random variable, then the kernel density approximation of its probability density function is:

$$\hat{f}_h\ (x) = \frac{1}{nh}\sum_{i=1}^{n} K\ \left(\frac{x - x_i}{h}\right)$$

Where K is some kernel and h is a smoothing parameter called the bandwidth. Quite often K is taken to be a standard Gaussian function with a mean of zero and a variance of 1. Thus, the variance is controlled indirectly through the parameter h:

$$K\left(\frac{x - x_i}{h}\right)\ = \frac{1}{\sqrt{2\pi}}e^{-\frac{(x-x_{i)}}{2h^2}}$$

Coefficients of correlation were estimated to examine the relationship between the mean values of cows' position (from GPS records) with the mean value of grazing preference (from behavioural observation). Cows' density (as % points per m^2) results for each season evaluated is presented in GIS layer.

5.4 RESULTS

5.4.1 Study One: Diet selection of dairy cows grazing a herb-clover mix pasture

Dairy cows actively selected chicory, plantain, and red clover (Si> 1), and appeared to avoid (Si< 1) white clover in all seasons evaluated (Figure 5-3). There was a three way interaction ($P<0.05$) between species, season, and grazing frequency for the selection index of chicory, plantain, red, and white clover in the herb-clover mix pasture. During late spring and summer, dairy cows selected chicory, plantain and red clover similarly regardless of the grazing frequency of two or four weeks. However, in autumn, the selection of red clover was lower ($P<0.05$) when swards were grazed every two weeks compared to plantain and chicory (Figure 5-3A). Whereas selection for plantain in the swards grazed every four weeks was lower ($P<0.05$) compared to selection for chicory and red clover in the same swards (Figure 5-3B). White clover was the species least selected ($P<0.05$) in all seasons evaluated in comparison to chicory, plantain, and red clover.

The selection of plantain increased ($P<0.05$) from late spring to autumn in the swards grazed every two weeks; however, when the swards were grazed every four weeks, selection increased from late spring to summer, and then declined in autumn, to levels similar to those observed in late spring. Selection for chicory increased ($P<0.05$) from late spring to summer and then remained at a similar level during autumn for both grazing frequencies. Selection of red clover in the swards grazed every four weeks increased ($P<0.05$) from late spring to autumn, whereas for the swards grazed every two weeks, selection for red clover was lower ($P<0.05$) in late spring and autumn than summer.

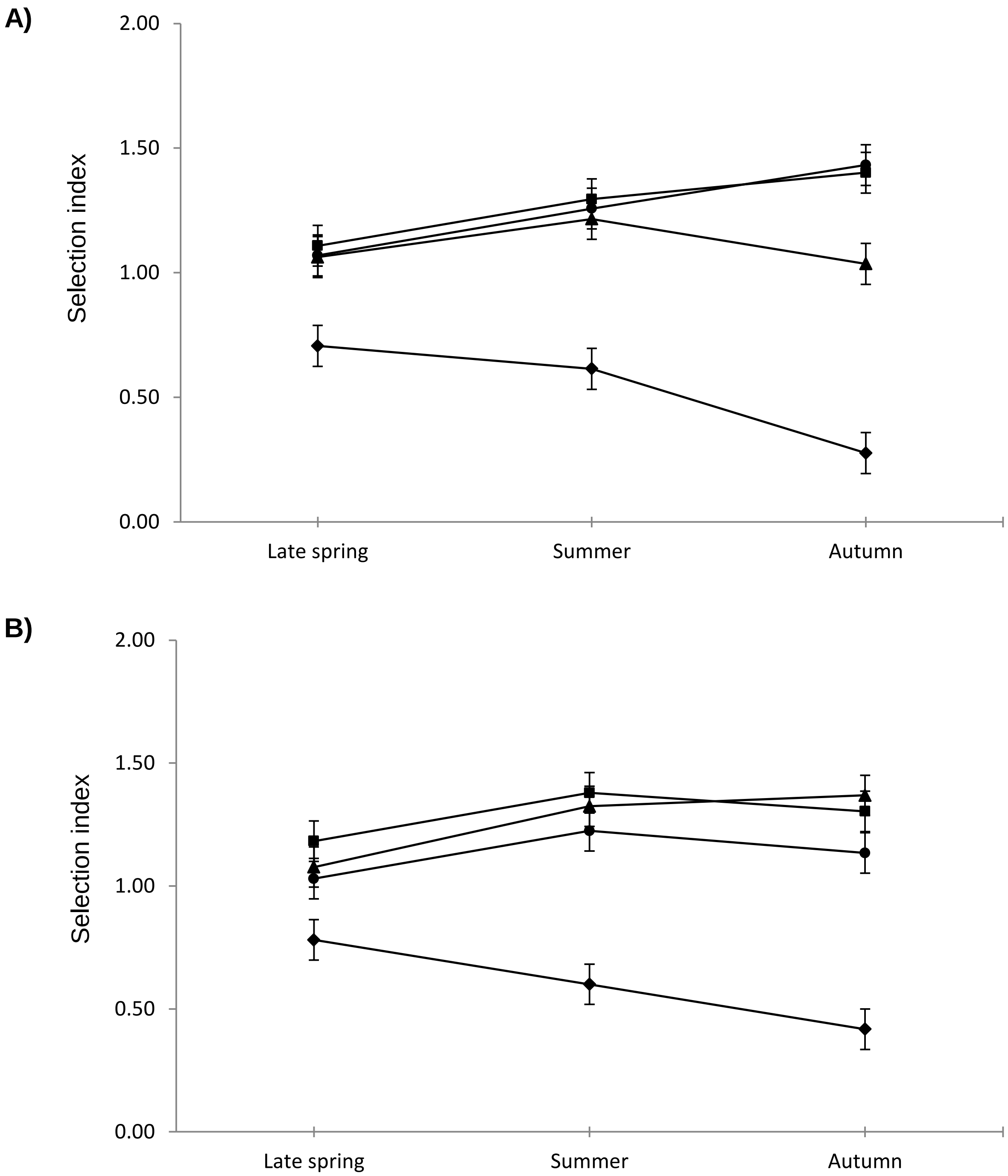

Figure 5-3 Selection index for chicory (■), plantain (●), red clover (▲) and white clover (♦) in a two-year old herb-clover mix pasture A) grazed every two or B) every four week frequencies by lactating dairy cows during late spring, summer and autumn Vertical bars represent the standard error of the mean (SEM).

5.4.1.1 Sward characteristics of the herb-clover mix pasture

The herb-clover mix had a greater (P<0.05) herbage mass when swards were grazed every four weeks compared to those swards grazed every two weeks in all three seasons (Table 5-2). There was no interaction (P>0.05) between grazing frequency and season on herbage mass in an herb-clover mix pasture.

Table 5-2 Herbage mass (kg DM/ha) of the herb-clover mix pasture grazed every two or every four weeks during late spring, summer and autumn (Mean ± SEM[1]).

	Grazing frequency	
Season	**2 week**	**4 week**
Late spring	2297 ± 167 [b]	4278 ± 191[a]
Summer	1786 ± 139 [b]	4309 ± 333 [a]
Autumn	2813 ± 426 [b]	4425 ± 359 [a]

[a, b] superscripts indicate values within rows that are significantly different (P<0.05)
[1] SEM, standard error of the mean

The botanical composition of the herb-clover mix pasture is presented in Figure 5-4. The proportion of plantain leaf in late spring (22%) and summer (31%) was similar (P>0.05) in both grazing frequencies; however, in autumn, the proportion of plantain leaf was greater (P<0.05) in the swards grazed every two weeks (52%) than in those grazed every four weeks (41%). In late spring, the swards grazed every four weeks had a greater (P<0.05) proportion of plantain stem (29%) compared to swards grazed every two weeks (8%), but the proportion of plantain stem in summer (4.7%) and autumn (0.6%) was lower (P<0.05) irrespective of grazing frequencies.

In late spring and summer, the herb-clover mix grazed every two weeks was dominated (P<0.05) by white clover (56% and 46%, respectively) compared to plantain, chicory (3% and 6%, respectively) and red clover (3% and 5%, respectively). In autumn, the proportion of white clover (21% of the sward) in the same swards was lower (P<0.05) than in late spring and summer. In all seasons, the swards grazed every four weeks had a lower (P<0.05) proportion of white clover (approximately 19% of the sward) than the swards grazed every two weeks.

The proportion of chicory in the sward was not affected (P>0.05) by grazing frequency in late spring (5% of the sward); but in summer and autumn, the proportion of chicory was greater (P<0.05) in the sward grazed every four weeks (13%) compared to swards grazed every two weeks (6% and 10%, respectively). In all three seasons the proportion of red clover was greater (P<0.05) in the swards grazed every four weeks (22%) compared to those grazed every two weeks (3%). There was also a lower (P<0.05) proportion of weeds in the swards grazed every four weeks compared to those swards grazed every two weeks.

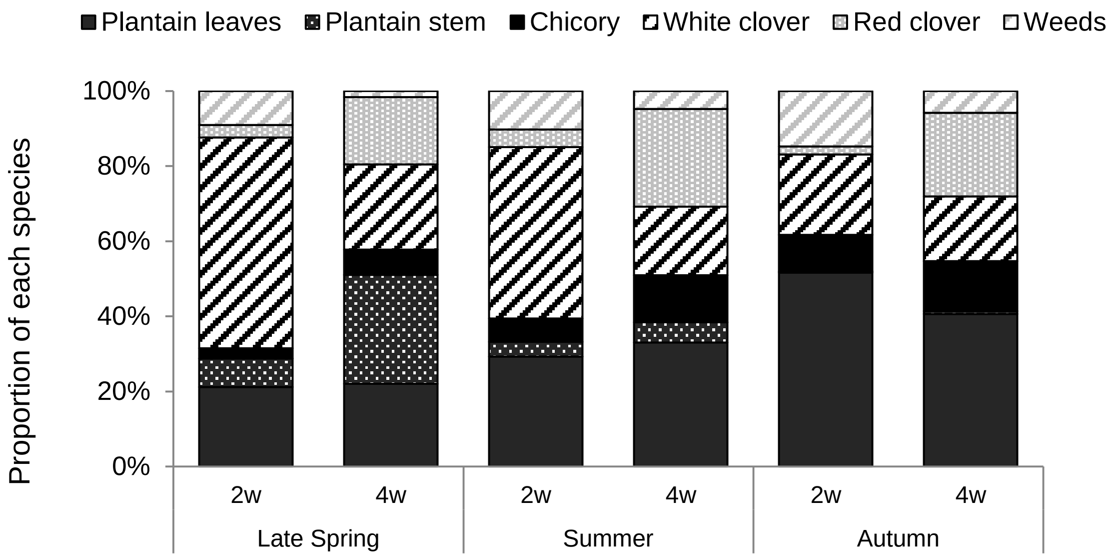

Figure 5-4 Botanical composition in the herb-clover mix pasture grazed every two (2w) and every four (4w) weeks during late spring, summer and autumn.

5.4.1.2 Sward structure of the herb-clover mix pasture

The botanical composition of the herb-clover mix swards when grazed every two or four weeks assessed at three different strata height (lower [0-7cm]; medium [7-20cm]; and upper [>20cm]) during late spring, summer and autumn are presented in Figure 5-5.

In late spring (Figure 5-5A), the upper (>20 cm) and middle (7-20 cm) strata were dominated by plantain (leaves + stem) compared to chicory, red clover, white clover, and weeds (Figure 5-5A). The upper strata (>20 cm) in the swards grazed every two weeks had a greater ($P<0.05$) proportion of plantain stem (76.6%) and a lower proportion of plantain leaf (12.6%) compared to those grazed every four weeks (52% and 17.8% plantain stem and leaf, respectively). The proportion of red clover (21.5%) and chicory (5.1%) were greater ($P<0.05$) in the swards grazed every four weeks compared with the swards grazed every two weeks (4.7% and 0%, for red clover and chicory, respectively). The proportion of white clover (2.2%) and weeds (2.3%) were similar ($P>0.05$) under both grazing frequencies (Figure 5-5A).

The middle strata (7-20 cm) had a lower ($P<0.05$) proportion of plantain stem and greater ($P<0.05$) proportion of plantain leaf and white clover compared to the upper strata (>20 cm), while the proportion of red clover and chicory remained similar ($P>0.05$) to the upper strata (Figure 5-5A). The proportion of plantain leaf in the middle strata (7-20 cm) was higher ($P<0.05$) in the two week grazing frequency (54.7%) than in the four week (30.5%) grazing frequency. However, the proportion of plantain stem (24.1%), chicory (8.7%), red (18.2%) and white clover (15.9%) were higher ($P<0.05$) in the swards grazed every four weeks compared to those swards grazed every two weeks (Figure 5-5A).

The lower strata (0-7 cm) in the swards grazed every two weeks was dominated by white clover compared to those swards grazed every four weeks. In late spring and summer, the lower strata (0-7 cm) in the swards grazed every two weeks had a higher (P<0.05) proportion of white clover compared to swards grazed every two weeks in autumn. Autumn swards grazed at both two and four week grazing frequencies had similar proportions of white clover.

During summer (Figure 5-5B), the proportion of plantain stem in the upper (>20 cm) and middle (7-20 cm) strata decreased (P<0.05) compared to late spring. The upper strata (>20 cm) in the swards grazed every two weeks contained plantain leaf (44.3%) and plantain stems (55.7%) in greater (P<0.05) proportions than the plantain leaf (18.8%) and plantain stems (11.9%) in swards grazed every four weeks. The proportion of red clover (40.7%), which was greater (P<0.05) than the proportion of chicory (9.7%), increased (P<0.05) in the upper strata (>20 cm) of the swards grazed every four weeks compared to the same sward in late spring; whereas, the proportion of white clover (2.9%) and weeds (2.2%) was similar (P>0.05) to late spring. The middle strata (7-20 cm) of the sward had a greater (P<0.05) proportion of plantain leaf (48.5% and 38%) than plantain stem (14.5% and 8.8%) in both the two and four week grazing frequencies, respectively. There was more (P<0.05) chicory (9.7%), red clover (23%), and white clover (12.5%) in the swards grazed every four weeks than in those grazed every two weeks (7.5%, 10.3%, and 10.5% for chicory, red and white clover, respectively).

In autumn (Figure 5-5C), the sward height was less than 20 cm therefore were no species in the upper strata (>20 cm). The middle strata (7-20 cm) of the swards grazed every two weeks had a greater (P<0.05) proportion of plantain leaf (81.8%) compared to the swards grazed every four weeks (49.6%). The proportion of chicory (15.9%) and red clover (0.4%) was lower (P<0.05) in the two weeks than in the every four weeks grazing frequency (chicory and red clover both 23%). In autumn, the proportion of plantain leaf in the middle strata (7-20 cm) was greater (P<0.05) than in late spring and summer for both grazing frequencies.

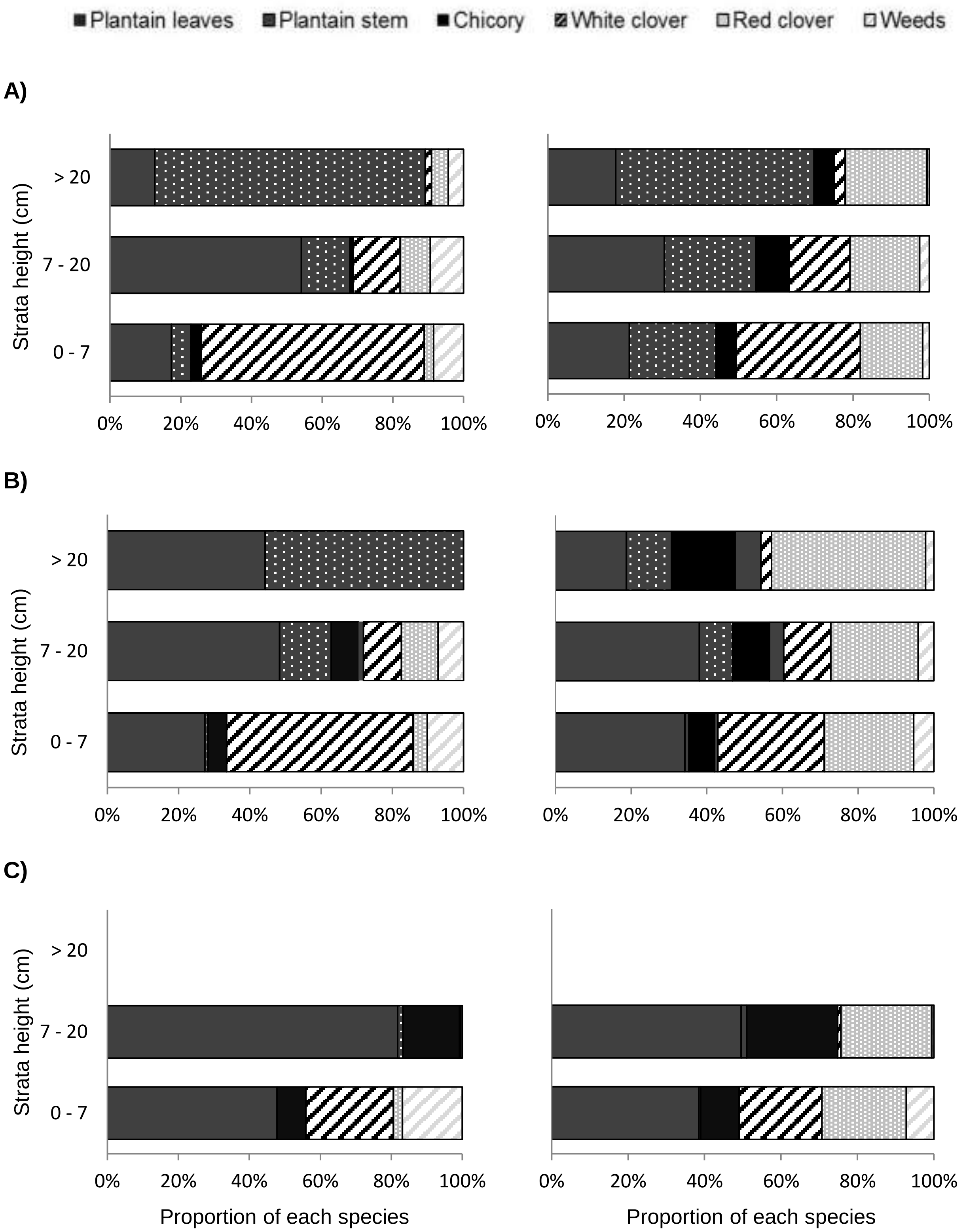

Figure 5-5 Botanical composition (%) in the herb-clover mix during A) late spring, B) summer, and C) autumn when grazing every two (left) or every four weeks (right) in the lover (<7 cm), middle (7-20 cm) and upper (>20 cm) strata height.

5.4.2 Study Two: Grazing preference of dairy cows for chicory, plantain and herb-clover mix pastures

5.4.2.1 Behavioural activity

The proportion of time that dairy cows spent grazing, ruminating, and idling was affected (P<0.05) by time of grazing (AM/PM). Dairy cows spent more (P<0.05) time grazing in the AM than in the PM, whereas in the PM more (P<0.05) time was spent ruminating and idling than in the AM (Table5-3). There was no significant difference (P>0.05) in time spent walking and drinking between time of grazing. There was no interaction (P>0.05) between time of grazing and season in relation to dairy cows activity.

Table 5-3 Percentage (%) of time spent in each behaviour activity during the morning (AM) and afternoon (PM) grazing times by dairy cows (mean ± SEM[1])

	Grazing time	
Activity	**AM**	**PM**
Grazing	93.0 ± 1.64 [a]	76.9 ± 2.35 [b]
Ruminating	1.9 ± 0.81 [b]	13.7 ± 1.54[a]
Idling	2.9 ± 0.84 [b]	7.0 ± 1.08 [a]
Walking	1.8 ± 0.36	1.7 ± 0.19
Drinking	0.4 ±0.04	0.7± 0.18

[a, b] superscripts indicate values within rows that are significantly different (P<0.05)

[1] SEM, standard error of the mean

5.4.2.2 Grazing preference

The grazing preference for chicory, plantain and herb-clover mix pastures of dairy cows is presented in Figure 5-6. In summer, the dairy cow grazing preference for the herb-clover mix (25.1%) was similar (P>0.05) to that of chicory (30.2%) and both were greater (P<0.05) than plantain (11.3%) in the pastures grazed every four weeks (Figure 5-6). In summer, the grazing preference for plantain grazed every four weeks was similar (P>0.05) to the preference for chicory (14.5%), plantain (9.7%), and the herb-clover mix (9.1%) pastures grazed every two weeks (Figure 5-6). In early autumn, the grazing preference for the herb-clover mix (41.4%) was greater (P>0.05) than the preference for both chicory and plantain (23.8%) in swards grazed every four weeks. In late autumn, the preference for the herb-clover mix (38.5%) was greater (P<0.05) than for plantain (28.6%) and both were greater (P<0.05) than chicory (16.5%). The time of grazing (AM or PM) had no effect (P>0.05) on the grazing preference of dairy cows for chicory, plantain or the herb-clover mix pastures.

In summer, cows had a lower (P<0.05) grazing preference for the herb-clover mix compared to late and early autumn. The preference for chicory in summer and early autumn was similar (P>0.05), but this declined (P<0.05) in late autumn. In contrast, the preference for the plantain increased (P<0.05) progressively from summer to late autumn.

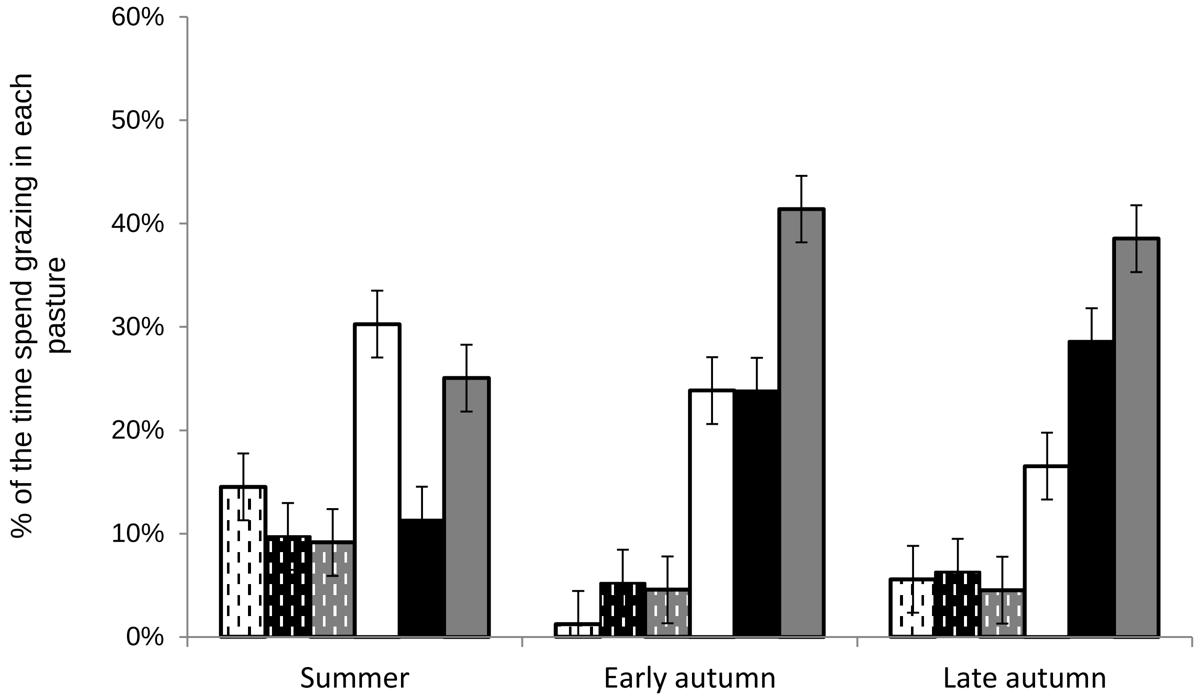

Figure 5-6 Dairy cow preference as the percentage of the time spent grazing each chicory (white), plantain (black), and herb-clover mix (grey) pasture type when grazed every two (dashed vertical) or every four weeks (solid fill) during summer, early and late autumn. Vertical bars represent the standard error of the mean (SEM).

5.4.2.3 Sward characteristics of chicory, plantain, and herb-clover mix pastures

The herbage mass (kg DM/ha) in the chicory, plantain, and herb-clover mix pastures is presented in Table 5-4. The herbage mass was greater ($P<0.05$) in the herb-clover mix pasture compared to the pure swards of chicory and plantain in the swards grazed every four weeks, while all pasture treatments grazed every two weeks had a similar ($P>0.05$) herbage mass.

The herbage mass of the pasture treatments grazed every four weeks was similar (P>0.05) in summer and early autumn, but decreased (P<0.05) in late autumn. When the pasture treatments were grazed every two weeks, the herbage mass decreased (P<0.05) from summer to late autumn. There was no interaction (P>0.05) between pasture treatment and season or between pasture treatment, grazing frequency and season on herbage mass in the chicory, plantain and herb-clover mix pastures.

Table 5-4 Herbage mass (kg DM/ha) in the chicory, plantain and herb-clover pastures and in summer, early and late autumn when grazed every two and every four weeks (Mean ± SEM[1])

	Grazing frequency	
	2 weeks	**4 weeks**
Pasture treatments		
Chicory	2523 ± 143 [c]	3408 ± 172 [b]
Plantain	2317 ± 137 [c]	3592 ± 167 [b]
Herb-clover mix	2371 ± 116 [c]	4366 ± 177 [a]
Season		
Summer	2913 ± 67 [b]	4061 ± 113 [a]
Early autumn	2319 ± 155 [c]	4388 ± 190 [a]
Late autumn	1979 ± 111 [d]	2917 ± 142 [b]

[a, b, c, d] superscripts indicate values within each variable and across grazing frequency that are significantly different (P<0.05)

[1] SEM, standard error of the mean

The botanical composition in the pasture treatments as the herbage mass from each species is presented in Figure 5-7, illustrating the herbage mass contribution (kg DM/ha) of various pasture components (chicory, plantain, white clover, red clover, weeds, and dead material) to the total herbage mass.

The pure chicory swards (Figure 5-7A) grazed every four weeks had higher ($P<0.05$) chicory mass in summer (1154 kg DM/ha) and early autumn (1242 kg DM/ha) than in late autumn (304 kg DM/ha). In the swards grazed every two weeks the chicory mass was lower ($P<0.05$) than (253, 143, and 40 kg DM/ha in summer, early and late autumn, respectively) than that grazed very four weeks. Grazing chicory every two weeks resulted in an increase ($P<0.05$) in white clover mass and a decrease ($P<0.05$) in dead material ($P<0.05$) compared to those swards grazed every four weeks.

The pure plantain swards (Figure 5-7B) had a greater ($P<0.05$) plantain mass than the chicory mass in the pure chicory swards (Figure 5-7A). The plantain mass was greater ($P<0.05$) in the swards grazed every four weeks (1334 kg DM/ha) compared to those grazed every two weeks (627 kg DM/ha) and remained similar ($P>0.05$) across seasons under both grazing frequencies. The white clover mass and dead material were greater ($P<0.05$) in the swards grazed every four weeks compared to those grazed every two weeks.

The herb-clover mix pasture (Figure 5-7C) had a greater ($P<0.05$) mass of plantain than chicory. The plantain mass in the swards grazed every four weeks was greater ($P<0.05$) in summer and late autumn (1450 and 1045 kg DM/ha, respectively) than in early autumn (605 kg DM/ha) while the contribution of chicory mass (435 kg DM/ha) was similar ($P>0.05$) over seasons.

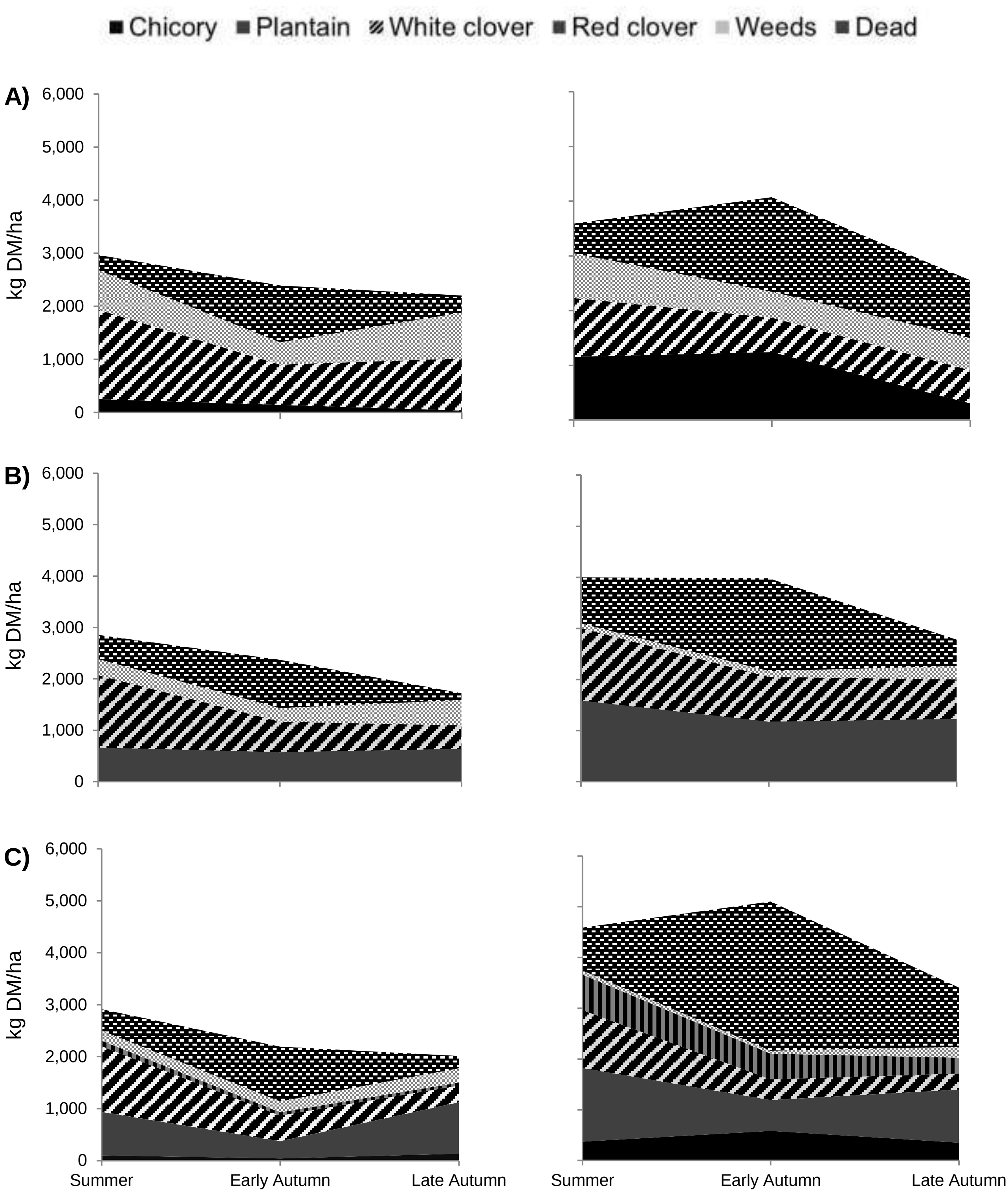

Figure 5-7 Herbage mass contribution (kg DM/ha) of different pasture components in A) chicory, B) plantain, and C) the herb-clover mix pastures grazed every two (left) and every four (right) week frequencies during summer, early and late autumn.

5.4.2.4 Relationship between GPS recording and grazing preference

The GPS data set comprised 4345, 4375, and 4871 records during summer, early autumn, and late autumn, respectively. Cows' density estimation indicated a heterogeneous intensity of cows' location within the pasture treatments. Intensity ranged from 0 to 0.25 cows per plot area (300 m^2) during the AM grazing and 0 to 0.20 cows per plot area (300 m^2) during the PM grazing.

There was a significant ($P<0.01$) relationship between GPS position and grazing preference of dairy cows for the pasture treatments in paddocks I, II and III. The correlation was positive and stronger in the trial paddocks that were grazed during the AM (I and III) period (Figure 5-8A and B) than in paddocks II, which was grazed during the PM period (Figure 5-8C). The GPS position was not significantly related ($P>0.05$) with the grazing preference of dairy cows for the pasture treatments in paddock IV (Figure 5-8D) likely to the tree bounder next to this paddock. Therefore, the overall correlation (Figure 5-8E) between GPS position and the grazing preference of dairy cows in the pasture treatments excluded paddock IV.

The estimated GPS point density is presented in a GIS layer for summer (Figure 5-9), early autumn (Figure 5-10), and late autumn (Figure 5-11) using the kernel density estimation.

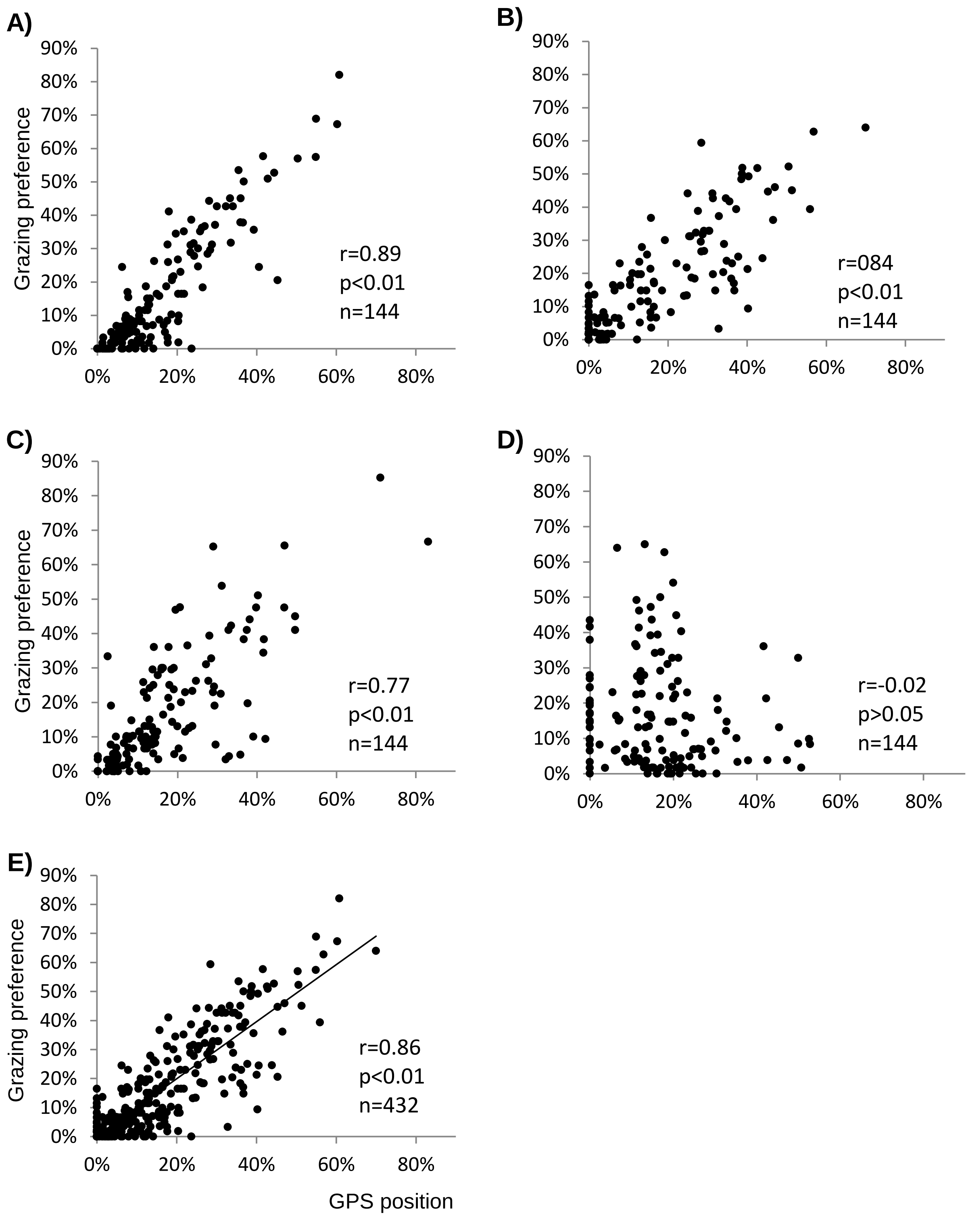

Figure 5-8. Relationship between GPS recording of cows position and grazing preference of dairy cows during the morning grazing (AM) in the A) paddock I and B) paddock III and during the afternoon grazing (PM) in the C) paddock II and D) paddock IV, and E) the overall relationship, when the paddock IV was excluded.

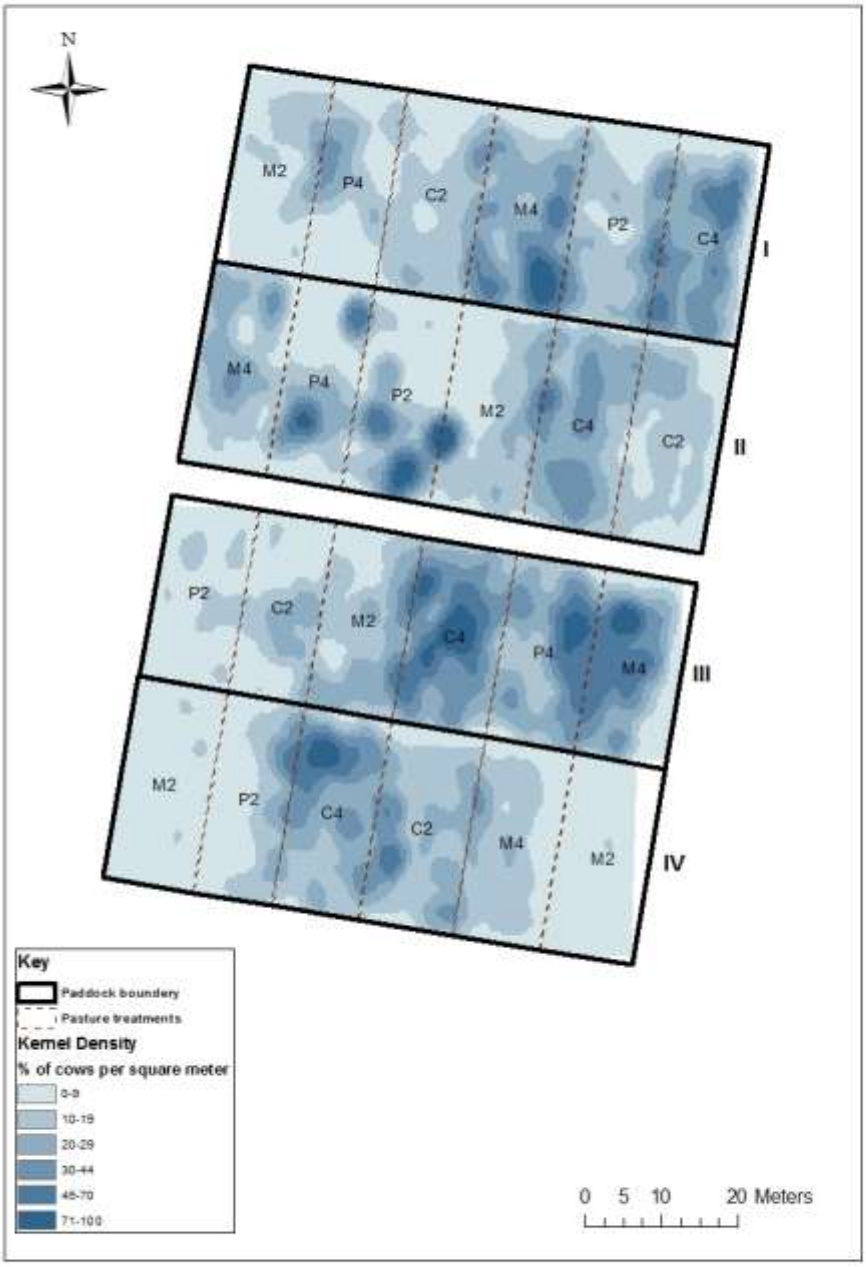

Figure 5-9 Kernel density estimated for cows position as the percentage of cows per square meter in the pasture treatments within paddock (I – IV) during summer. C2 = chicory grazed every two weeks, C4 = chicory grazed every four weeks, P2 = plantain grazed every two weeks, P4 = plantain grazed every four weeks, M2 = herb-clover mix grazed every two weeks, and M4 = herb-clover mix grazed every four weeks.

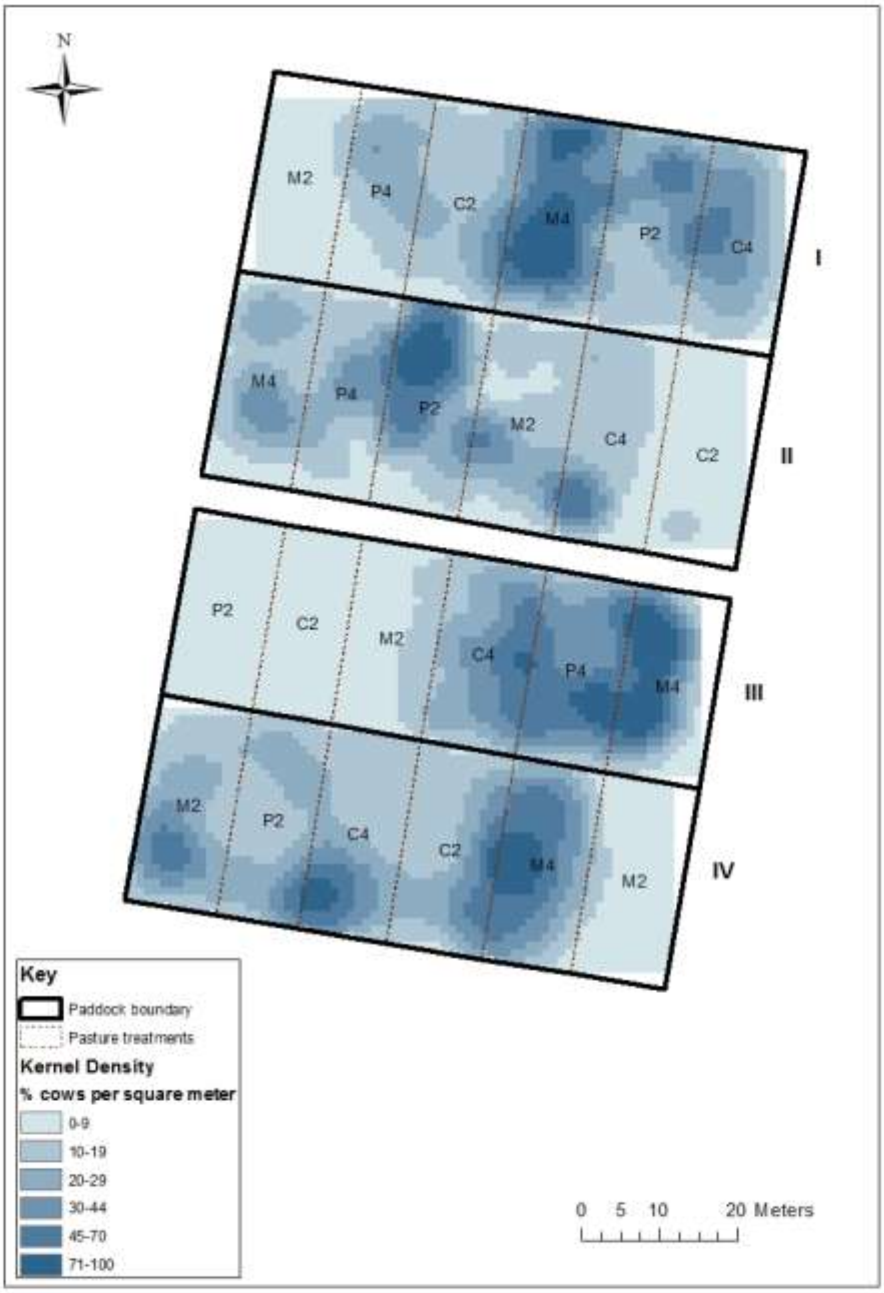

Figure 5-10 Kernel density estimated for cows position as the percentage of cows per square meter in the pasture treatments within paddock (I – IV) during early autumn. C2 = chicory grazed every two weeks, C4 = chicory grazed every four weeks, P2 = plantain grazed every two weeks, P4 = plantain grazed every four weeks, M2 = herb-clover mix grazed every two weeks, and M4 = herb-clover mix grazed every four weeks.

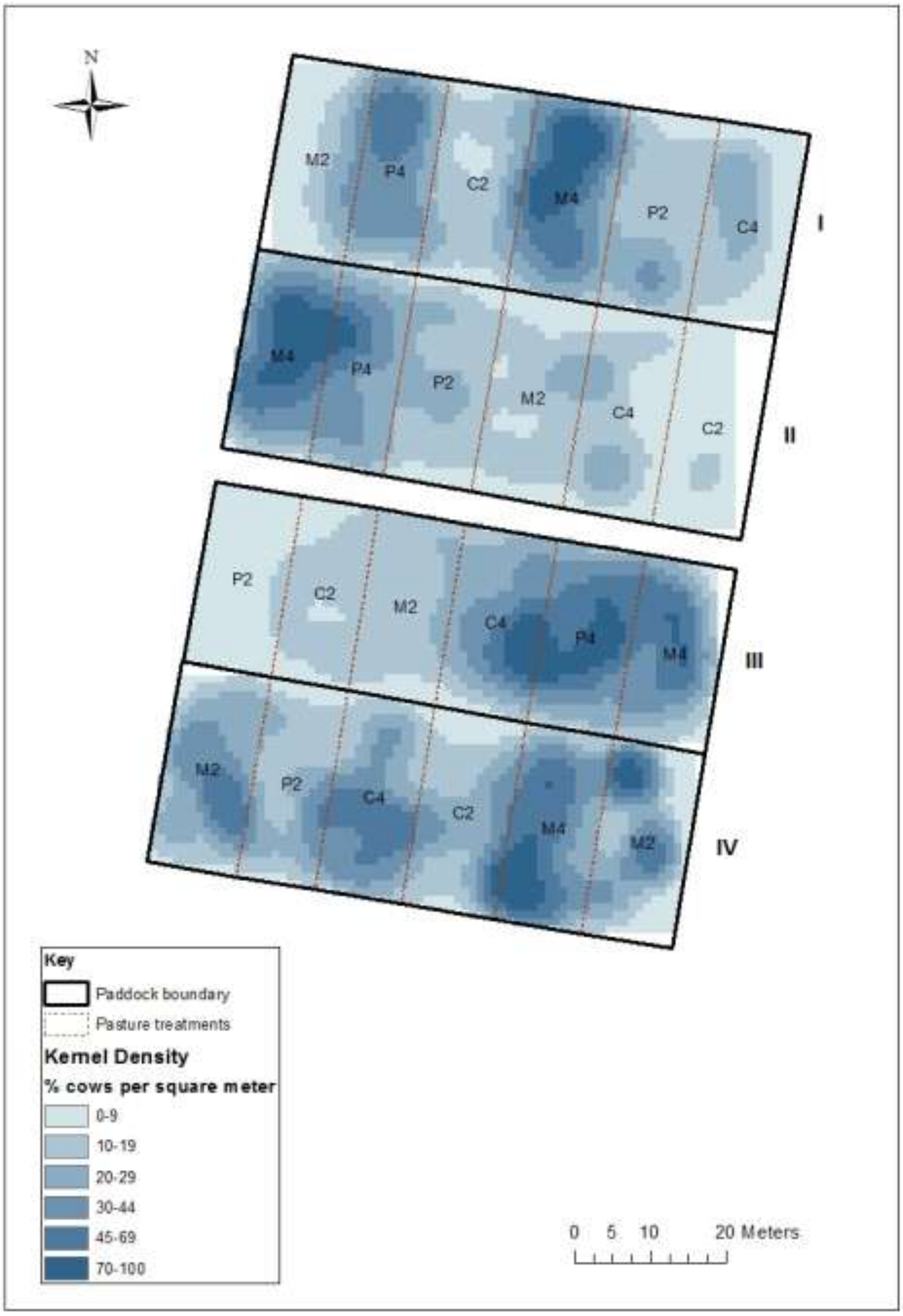

Figure 5-11 Kernel density estimated for cows position as the percentage of cows per square meter in the pasture treatments within paddock (I – IV) during late autumn. C2 = chicory grazed every two weeks, C4 = chicory grazed every four weeks, P2 = plantain grazed every two weeks, P4 = plantain grazed every four weeks, M2 = herb-clover mix grazed every two weeks, and M4 = herb-clover mix grazed every four weeks.

5.5 DISCUSSION

Study One showed that in a herb-clover mix pasture dairy cows positively selected (Si> 1) for chicory, plantain and red clover; while white clover was always the least selected (Si< 1) species across all seasons, regardless of grazing frequency (Figure 5-3). Grazing animals select their diet by removing some sward components, either plant parts or whole plants, in preference to others on offer (Hodgson, 1979; Parsons et al., 1991). Although plant palatability influences the acceptance of a species in preference to others on offer (Arnold, 1964; Heady, 1964; Hodgson, 1979), the diet selection of grazing animals can be influenced by variation in vegetation characteristics of the swards. Both accessibility (vertical availability) and availability (horizontal availability) of the species in the sward and describing the sward in layers is useful when examining the diet selection of grazing animals (Dumont, 1997; Edwards et al., 1996; Hodgson et al., 1997; Parsons et al., 1994; Ungar and Noy-Meir, 1988).

During late spring and summer, the diet selection of dairy cows for chicory, plantain, and red clover was similar (Figure 5-3). Red clover has been reported to be preferred by lambs over chicory and plantain (Cave et al., 2015; Pain et al., 2014; Pain et al., 2010). In the current study dairy cows indiscriminately selected these species in the herb-clover mix. The high preference and palatability of red clover has been widely recognised and attributed to high crude protein (CP) concentration and rapid rumen clearance compared to perennial ryegrass (Speijers et al., 2004). Similarly, previous research has reported that chicory and plantain are also rapidly degraded in the rumen with lower rumen retention time (Burke et al., 2000; Hoskin et al., 1995; Kusmartono et al., 1997). Dairy cows grazing chicory or plantain have showed greater mastication and swallowed smaller particles, reducing their rumination time compared to cows grazing perennial ryegrass (Gregorini et al., 2013). Lower rumen retention and faster rumen clearance of chicory, plantain and red clover suggested a high palatability and higher intake potential in dairy cows (Barry, 1998; Burke et al., 2000; Stewart, 1996).

Diet selection has been positively related to the RA of the species in the sward (Davison et al., 1997; Dumont et al., 2007; Hernández et al., 1995). A higher selection of chicory and plantain by lambs over red and white clover was associated with a greater proportion of chicory and plantain in the middle (4-8 cm) and upper strata (>8 cm) of the sward during early spring (Cave et al., 2015). The herbage consumed by animals is typically of similar composition to the botanical composition of the surface horizons (Hodgson, 1990; Milne et al., 1982). Nevertheless, in this study, dairy cows selected these species independently of their proportion in the middle (7-20 cm) and upper (>20 cm) strata in the swards in late spring and summer. This suggests that dairy cows, grazing a herb-clover mix pasture, will indiscriminately select these species when they are present in the grazed horizon (>7 cm) of the sward (Figure 5-5A and B) (Hodgson, 1990).

In contrast, white clover was the species least selected (Si< 1) by dairy cows. White clover is known to be highly palatable and actively selected by cows (Rutter et al., 2004a; b). However, the diet selection of a preferred species may decline as its availability in the sward decreases and, therefore, the rate at which animals encounter them also declines (Edwards et al., 1996; Parsons et al., 1991). In Study One, therefore, the selection against white clover may have been a consequence of its poor accessibility (vertical availability) in the sward, as the greater proportion of white clover was found in the lower strata (<7 cm) of the sward (Figure 5-5). The diet selection for white clover may be increased by increasing the proportion of white clover in grazed horizon (>7 cm) of the sward (Milne et al., 1982).

The diet selection of chicory and plantain increased from late spring to summer (Figure 5-3). This seasonal change in the diet selection of chicory and plantain appeared to be influenced by the vertical accessibility of these species in the grazed horizon (>7 cm) of the swards. Although the diet selection of chicory was consistently high across all seasons irrespective of grazing frequency (every two or every four weeks), the higher diet selection for chicory and plantain in summer is likely a consequence of the lower proportion of plantain stem in the upper strata (>20 cm) of the sward in summer compared to late spring (Figure 5-5). Stem development of the plant have been associated with palatability problems for lambs grazing plantain during late summer and early autumn (Fraser and Rowarth, 1996; Swainson and Hoskin, 2006). Stems are reported to be a vertical barrier to bite depth in swards (Benvenutti et al., 2006), and animals select against reproductive areas and increase their preference for a vegetative sward (Dumont et al., 2007; Ginane et al., 2003). Consequently, the diet selection of both herbs can be increased by improving the accessibility of chicory and plantain leaves in the swards.

Study Two showed that of the swards grazed every four weeks dairy cows preferred the herb-clover mix pastures, rather than pure swards of chicory or plantain (Figure 5-6). Dairy cows avoided swards grazed every two weeks with lower herbage masses and preferred those grazed every four weeks that had higher herbage masses (Distel et al., 1995). Similarly, the GPS recording of cow position was positively related with the time that cows spent grazing each of the pastures treatments, except for the paddock IV (Figure 5-8). The lack of correlation in paddock IV was likely due to the tree border located next to the paddock (Rempel and Rodgers, 1997). The kernel density estimated from GPS position records indicated that the dairy cows spent more time on the pasture treatments grazed every four weeks and avoided those grazed every two weeks (Figure 5-9 to 5-10). Grazing animals prefer swards that they can eat quickly in order to maximise their herbage intake (Black and Kenney, 1984). High herbage masses allow a greater DMI by dairy cows is possible at compared to low herbage masses (Forbes and Hodgson, 1985; Orr et al., 2005). Both cattle and sheep have been reported to actively prefer a mixed diet compared to a mono-species if it is readily available (Parsons et al., 1994; Rutter et al., 2004a). The current study may suggest that the greater herbage mass in the herb-clover mix, containing highly palatable species would allow cows to obtain a higher DMI from herb-clover mix pastures than chicory and plantain (Table 5-3).

The grazing preference of dairy cows for chicory and plantain varied between seasons (Figure 5-6). This seasonal effect on the preference of chicory and plantain by dairy cows may be due to changes in the physical and chemical characteristics of the pastures across the seasons (Hodgson et al., 1997; Horadagoda et al., 2009). During summer and early autumn, dairy cows maintained their grazing preference for chicory, but this declined in late autumn. Previous work has reported that chicory maintains a metabolisable energy (ME) content higher and more stable throughout seasons than perennial ryegrass and plantain (Barry, 1998). The greater herbage mass from chicory in summer and early autumn than in late autumn in the swards grazed every four weeks, with a greater ME content (reported previously in chapter 4) may have maintained the grazing preference for chicory; however, the lower herbage mass from chicory in late autumn caused decline of its grazing preference (Figure 5-7).

The grazing preference of dairy cows for plantain increased from summer to late autumn, despite similar herbage masses in the swards. The palatability of a plant can decrease due to an increase in lignin concentration making it less palatable for animals (Baumont et al., 2000). The lignin concentration in plantain declined from late spring to summer and its ME content increased (reported previously in Chapter 4; Table 4-1). This may explain the increasing grazing preference for plantain from summer to late autumn (Figure 5-6). Cave et al. (2015), however, suggested that the poor palatability of plantain during autumn may have been due to high concentrations of bioactive compounds (aucubin and acteoside), which have been reported to peak during autumn in plantain swards (Tamura and Nishibe, 2002). The present study showed that the grazing preference for plantain was higher in autumn than summer. This likely suggests that bioactive compounds present in plantain did not affect dairy cow preference of plantain, and that the differences between seasons may have been due to a greater proportion of plantain stem in the swards.

5.6 CONCLUSIONS

Dairy cows displayed a greater grazing preference for the herb-clover mix rather than for chicory and plantain, from swards grazed every four weeks than those grazed every two weeks. This is likely due to the greater herbage mass available in the herb-clover mix grazed at four week intervals. Dairy cows grazing the herb-clover mix pasture indiscriminately selected chicory, plantain, and red clover from the grazed horizon (>7 cm) and avoided white clover due its greater proportion in the lower strata (<7 cm) which reduced its accessibility. The diet selection for chicory and plantain for dairy cows changed across seasons due to the availability and accessibility of the species in the swards. The diet selection of chicory was consistently high in all seasons. The higher herbage masses during summer and early autumn resulted in high grazing preference, but in late autumn, the lower chicory mass caused a decline in dairy cow grazing preference. The diet selection and grazing preference for plantain increased from late spring to autumn which was likely due to the lower proportion of plantain stems in the swards thus improving the palatability of plantain.

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6 BIOACTIVE COMPOUNDS, AUCUBIN AND ACTEOSIDE, IN PLANTAIN AND THEIR EFFECT ON *in vitro* RUMEN FERMENTATION

6.1 ABSTRACT

Plantain (*Plantago lanceolata* L.) contains bioactive compounds with antimicrobial activity that can influence ruminal fermentation. This study aimed to identify the concentration of catalpol, aucubin, and acteoside in plantain cv. 'Ceres Tonic' through two growing seasons (2011-2012 and 2012-2013). Then the herbage with highest levels of bioactive compounds was used to evaluate their effect on rumen *in vitro* fermentation. Tonic plantain had almost nil concentration of catalpol. Nevertheless, the concentration of aucubin was lower ($P<0.05$) than acteoside in both growing seasons. Both aucubin and acteoside concentrations increased ($P<0.05$) through the growing season, from 1.78 to 3.80 mg/g DM in the first and from 0.44 to 6.87 mg/g DM in the second growing seasons; while, acteoside increased from 23.6 to 35.4 mg/g DM and from 0.5 to 41.7 mg/g DM. The *in vitro* experiment evaluated the effect of aucubin (au) and acteoside (ac) on ammonia (NH_3), volatile fatty acid (VFA) and gas production (GP). Aucubin and acteoside were added to chicory as negative and to plantain as positive controls. The treatments were (i) chicory (CH); chicory+10 mg aucubin/g DM (CH+10au); (ii) chicory+20 mg aucubin/g DM (CH+20au); (iii) chicory+40 mg acteoside/g DM (CH+40ac); (iv) plantain naturally containing 7 mg/g DM of aucubin 36 mg/g DM of acteoside (PL); (vi) plantain+extra 10 mg aucubin /g DM (PL+10au); and (vii) plantain+extra 36 mg acteoside/g DM (PL+36ac). Chicory fermentation produced more ($P<0.05$) NH_3, acetate and total VFA, while propionate production was similar ($P>0.05$) to that of plantain with its natural levels of aucubin and acteoside. The exogenous addition of both compounds reduced ($P<0.05$) net NH_3 production of chicory and plantain fermentations. The addition of aucubin reduced ($P<0.05$) acetate and total VFA production, potentially due to its bactericide activity, while the greater ($P<0.05$) propionate and total VFA production with the addition of acteoside suggests its use as an energy source. Therefore, acteoside would have greater positive animal effect over aucubin.

Keywords: Plantain, aucubin, acteoside, *in vitro* fermentation, ammonia, volatile fatty acids.

6.2 INTRODUCTION

Plantain (*Plantago lanceolata* L.) is becoming widely used by farmers to supplement dairy cows over the summer/autumn period. Plantain is an alternative perennial herb that establishes rapidly, is productive in a wide range of agricultural soils, is drought tolerant and accumulates high herbage mass over spring, summer and autumn (Lee et al., 2015; Powell et al., 2007; Moorhead, et al., 2002). However, plantain also contains biologically active compounds; the most well-known are the iridoid glycosides: aucubin and catalpol; and the phenylpropanoid glycoside acteoside (Syn. verbascoside) (Tamura and Nishibe, 2002; Stewart, 1996). These compounds have been reported with antimicrobial and antifungal effects (Kim et al., 2000; Davini et al., 1986; Andary et al., 1982), and it has been suggested that these compounds, which are present in plantain, may influence the rumen micro flora of grazing ruminants and ultimately their nutrient utilisation (Moorhead et al., 2002; Burke et al., 2006; Swainson and Hoskin, 2006); however, their impact on rumen fermentation is unclear.

Ammonia (NH_3) concentration in the rumen fluid of deer was five-fold less when plantain was fed to deer compared to perennial ryegrass (*Lolium perenne* L.) (Swainson and Hoskin, 2006). Additionally, a lower nitrogen (N) concentration in the urine was obtained on cows grazing diverse pasture which included plantain, but it was not determined if this effect was by improvement of N efficiency or N dilution in the urine (Totty et al., 2013). Greater urine flows have been recorded in animals grazing plantain (Wilman and Derrick, 1994), consistent with the diuretic effect of iridoid glycosides (Tamura and Nishibe, 2002). Secondary compounds have several mechanisms that can reduce NH_3 concentration in the rumen, such as a reduction in peptidolysis and deamination, due to direct inhibition of rumen microbial growth, or an inhibition of rumen hyper-ammonia producing bacteria (Durmic and Blade, 2012).

The presence of these compounds in plantain capable of affecting the rumen fermentation process is likely to have important implications for rumen N efficiency (Stewart, 1996). Reducing excess NH_3 concentration is desirable because it minimises N losses to the environment (Attwood et al., 1998). Ammonia is either used by rumen bacteria or absorbed into the blood stream. Once absorbed, the NH_3 may be recycled or converted into urea and excreted, mainly in the urine (Pacheco and Waghorn, 2008). Urine N is immediately available for leaching and volatilisation resulting in significant nitrous oxide (N_2O) emissions. Reducing the N excreted in the urine is considered a method to increase the efficiency of N utilisation in ruminants.

The antimicrobial effect of aucubin has been well documented (Kim et al., 2000; Danvini et al., 1986; Bartholomaeus and Ahokas, 1995) but not for acteoside (Anadary et al., 1982) and the effect of these secondary compounds on rumen fermentation appear not to have been assessed. The hypothesis was that the bioactive compounds in plantain will decrease NH_3 production and affect volatile fatty acids (VFA) production in the rumen. This study aimed to examine the concentration of these bioactive compounds in plantain cv. ‘Ceres Tonic’ and evaluate the *in vitro* fermentation of plantain and compare it to chicory (perennial herb known not to contain these bioactive compounds) by the exogenous addition of aucubin and acteoside.

6.3 MATERIAL AND METHODS

6.3.1 Plant material

The plantain (cv. ‘Ceres Tonic’) and chicory (cv. ‘Grassland Choice’) evaluated in this study were collected from pure swards established in October, 2011 and managed with two grazing frequencies (two and four weeks) throughout two growing seasons (2011-2012 and 2012-2013). The plantain and chicory pastures were in a randomised complete block design (n=5) with a factorial arrangement of the pasture. The day before grazing one grab sample (100 g fresh weight) was taken from each plot of the plantain and chicory (five samples per pasture). The samples were taken in December, 2011 and May, 2012 (in the first growing season) and in October, 2012, January, 2013, and May, 2013 (in the second growing season) and stored at -20 °C until later analysis.

6.3.1.1 Laboratory analysis of secondary compound

Catalpol, aucubin and acteoside in plantain and chicory were determined by high-performed liquid chromatography (HPLC) by following method of Tamura and Nibishe (2002). The samples stored at -20 °C were freeze-dried and ground to pass through a 1 mm diameter sieve. A 100 mg aliquot from each of the grounded samples was taken for extraction of aucubin, catalpol, and acteoside, with 10 mL of methanol (MeOH) in 15 mL tubes and shaken for 2 h at room temperature. The solid plant material was filtered out using grade 41 quantitative filter papers (Whatman Co., Ltd., England). Then, 2 mL of the filtrate was diluted in 8 mL of ultra-pure water, and further filtered using 0.2 µm syringe filters (Whatman Co., Ltd., England) and a 20 µL of aliquot used for HPLC analysis for the simultaneous determination of catalpol and aucubin. Whereas for acteoside, 2 mL of the filtrate undiluted was filtered using 0.2 µm syringe filter (Whatman Co., Ltd., England) and a 20 µL of aliquot used for HPLC analysis.

Commercially available catalpol, aucubin, and acteoside (99% pure; Extrasynthese S.A, France) were used as standards. The standard solution contained 2 mg each of catalpol and aucubin in 50 mL of 20% MeOH and 1 mg of acteoside in 5 mL of pure MeOH. HPLC was performed at 40 °C using a 100 mm x 6.0 YMC pack ODS-A column protected by a YMC guard pack. The mobile phase was 1% acetronitrile in water for catalpol and aucubin and 29% MeOH in water (containing 5% acetic acid) for acteoside. The flow rate was 1 mL/min. For catalpol and aucubin, a wavelength detector was performed at 240 nm and at 330 nm for acteoside. The HPLC system consisted on a Dionex UltiMate 3000 HPLC system equipped with an UltiMate 3000 Pump, an UltiMate 3000 Autosampler Column Compartment, an UltiMate 3000 variable wavelength detector and Chromeleon software (version 6.8) for data processing.

6.3.2 *In vitro* experimental design

The *in vitro* gas production technique was used to investigate the impact of aucubin (au) and acteoside (ac) (>90% and >99% pure, respectively; Extrasynthese S.A, France) on rumen fermentation characteristics at higher concentration to those found in plantain. Chicory, a herb forage, does not contain detectable levels of these compounds and so was used as a negative control substrate whereas plantain, naturally containing both compounds, was used as positive control.

The treatments evaluated were: (i) chicory negative control, 0 mg aucubin or acteoside/g DM (CH); (ii) chicory plus10 mg aucubin/g DM (CH+10au); (iii) chicory plus 20 mg aucubin/g DM (CH+20au); (iv) chicory plus 40 mg acteoside/g DM (CH+40ac); (v) plantain positive control with existing natural levels of aucubin (7 mg/g DM) and acteoside (36 mg/g DM) (PL); (vi) plantain plus an additional 10 mg aucubin/g DM (PL+10au); and (vii) plantain plus an additional 36 mg acteoside/g DM (PL+36ac). The treatments are further summarised in Table 6-1.

Table 6-1 Summary of the chicory (CH) and plantain (PL) treatments and concentration (mg/g DM) of the aucubin (au) and acteoside (ac) tested.

		Aucubin and acteoside concentration (mg/g DM)		
Treatments	Substrate	natural level	level added	Total
CH	Chicory	0 aucubin	0 aucubin	0 aucubin
		0 acteoside	0 acteoside	0 acteoside
CH+10au	Chicory	0 aucubin	**10 aucubin**	10 aucubin
		0 acteoside	0 acteoside	0 acteoside
CH+20au	Chicory	0 aucubin	**20 aucubin**	20 aucubin
		0 acteoside	0 acteoside	0 acteoside
CH+40ac	Chicory	0 aucubin	0 aucubin	0 aucubin
		0 acteoside	**40 acteoside**	40 acteoside
PL	Plantain	7 aucubin	0 aucubin	7 aucubin
		36 acteoside	0 acteoside	36 acteoside
PL+10au	Plantain	7 aucubin	**10 aucubin**	17 aucubin
		36 acteoside	0 acteoside	36 acteoside
PL+36ac	Plantain	7 aucubin	0 aucubin	7 aucubin
		36acteoside	**36 acteoside**	72 acteoside

6.3.2.1 In vitro incubations

The *in vitro* fermentation assay was undertaken over 24 h using an incubator with the capacity for 32 bottles (Contherm Scientific Limited). All treatments were incubated in two sets of duplicate bottles where one set of bottles was used to record gas production and the other set used to determine products of fermentation, NH_3 and VFA. The incubations were carried out on three occasions, evaluating three replicate herbage samples (derived from separate plots in the field, n=3).

The chicory and plantain used in this *in vitro* study as substrate were from the pastures grazed every four weeks and were harvested in May 2013. Approximately 300 mg DM of substrate (1 mm ground plantain and chicory) was weighed into a 125 mL serum bottle. Aucubin and acteoside were then added to the chicory and plantain bottles at the concentrations outlined in Table 6-1.

Three fistulated cows grazing perennial ryegrass pasture were used as rumen fluid donors. The rumen fluid obtained in the morning from multiple sites within the rumen and transferred to the laboratory in pre-warmed thermos flasks. Once in the laboratory, the rumen fluid was strained through a double layer of cheesecloth, pooled in equal proportions, and mixed with McDougal's buffer (Mould et al., 2005) at a 1:4 ratio, under continuous flushing with carbon dioxide (CO_2) in a water bath at 39 °C to obtain rumen fluid as the source of inoculum. Then, 30 mL of this buffered rumen fluid was injected into each bottle containing substrate plus or minus additional secondary compounds and sealed with rubber stoppers, manually agitated and place inside the incubator at 39 °C for 24 h.

6.3.2.2 Determination of gas production and end fermentation products

The headspace gas pressure inside each bottle was measured automatically using a pressure sensor connected to a needle in the cap of the incubation bottle (40PC015G1A, Honeywell, International Inc., Morris town, NJ, USA). Pressure was recorded every minute and when pressures exceeded 10 kPa the accumulated gas was released.

The set of bottles for NH_3 and VFA determination were repeat sampled at 1.5, 3, 5, 8, 12 and 24 h, taking 1.8 mL of medium from each bottle at each sampling time. The samples were transferred to micro tubes and centrifuged at 2100 x *g* for 10 min at 4 °C. Then, duplicate 0.9 mL aliquots of the supernatant were transferred into micro-tubes with 0.1 mL of an internal standard (19.87 mM ethyl butyric acid, 20% v/v ortho-phosphoric acid) and stored at -20 °C until later analysis of NH_3 and VFA.

6.3.2.3 Laboratory analysis for in vitro incubations

Plantain and chicory substrate were analysed for total dry matter (DM) determined by drying the samples at 105 °C in a conventional oven (AOAC, 2000; method 930.15, 925.10) and organic matter (OM) by ashing the samples for 16 h at 550 °C in a furnace (AOAC, 2000; method 942.05). Total N was determined by combustion using a Leco analyser (AOAC, 2000; method 968.06), and crude protein (CP) was calculated by multiplying the N content by 6.25. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were analysed in a Tecator Fibretec System (Foss Fibretec, Höganäs, Sweden) by the detergent procedures of Robertson and Van Soest (1981). Cellulose was calculated as ADF less lignin, and hemicellulose as NDF less ADF. Hot water soluble carbohydrates (HWSC) were measured by the reducing sugar method (Nelson, 1944). Organic matter digestibility (OMD) and metabolisable energy (ME) were measured by following the Roughan and Holland (1977) method

The samples for VFA and NH_3 analysis were thawed and centrifuged at 2100 x *g* for 10 min at 4 °C, and 0.8 mL of the supernatant was transferred into a crimp cap vial to determine individual VFA (acetate, propionate, butyrate, isobutyrate, and isovalerare) by gas chromatography (HP 6890, Santa Clara, CA, USA). Part of the final supernatant (approximately 0.1 mL) was used to determine NH_3 concentration by the colorimetric method described by Weatherburn (1967).

6.3.3 Calculations and statistical analysis

The gas production (GP) profiles of each bottle were fitted to the model described by Wang et al. (2011), using the following formula:

$$V = (A\ (1-\exp(-kt))/\ (1+\exp(\ln(1/d)-kt))$$

Where:

V: total gas produced at time t (mL/g DM)

A: is the asymptotic potential of GP (mL/g DM)

k= is the fractional rate gas production per hour (/h)

d= is a shape parameter indicating a sigmoidal or no sigmoidal shape of the gas curve.

The half time of when the potential GP was reached ($T^{1/2A}$) and the fermentation rate ($R^{1/2A}$) at $T^{1/2A}$ were calculated as follow:

$$T^{1/2A} = (\ln(2+1/d))/k$$

$$R^{1/2A} = (k(d+0.5))/(1+d)$$

Data were analysed using the PROC MIXED procedure of SAS 9.3 (SAS Institute, 2009). The concentration of catalpol, aucubin, and acteoside in plantain were analysed in a model that included the fixed effects harvest date and grazing frequency and their interactions with block included as random effect.

The model parameters (A, $T^{1/2A}$, $R^{1/2A}$ and V24h) and the fermentation end products after 24 h of incubation obtained from the *in vitro* study were analysed with incubation day treated as a random effect. Net NH_3 and individual VFA production over time were analysed in a design where treatments and time (1.5, 3, 5, 8, 12 and 24 h) and the interaction of treatment and time where fitted as fixed effects. The inclusion of incubation day as a random effect provided true replication. Means were compared using the least squares means test and significance was declared at $P<0.05$.

6.4 RESULTS

6.4.1 Secondary compounds in plantain

The concentration of secondary compounds found naturally occurring in the plantain samples harvested from the two growing seasons and two grazing frequencies are presented in Table 6-2. Catalpol was detected at very low concentrations, ranging from 0.01 to 0.06 mg/g DM, with no differences observed across the growing season, harvest dates or due to grazing frequency. Aucubin and acteoside concentrations increased ($P<0.05$) over the growing season in both years (Table 6-2). These secondary compounds were not detected in chicory, so data is not included.

There was an interaction ($P<0.05$) between grazing frequency and harvest date observed for aucubin concentration in the first growing season only; grazing every two weeks appeared to inhibit aucubin accumulation towards the end of the growing season (May). No effect of grazing frequency was observed in relation to acteoside concentration, and nor was any interaction observed during the second growing season.

Table 6-2 Concentration (mg/g DM) of catalpol, aucubin and acteoside in plantain (mean ± SEM[1]).

	Catalpol	Aucubin	Acteoside
First growing season (2011-2012)			
Grazing frequency			
2 weeks	0.03 ± 0.01	1.99 ± 0.29^b	25.71 ± 4.06
4 weeks	0.02 ± 0.01	3.59±0.89^a	33.31 ± 4.17
Date			
Dec-2011	0.03 ± 0.01	1.78± 0.24^b	23.61 ± 3.03^b
May-2012	0.02 ± 0.01	3.80± 0.80^a	35.40± 3.96^a
Grazing frequency * date			
2weeks*Dec-2011	0.05 ± 0.02	1.80 ± 0.35^c	20.59 ± 3.36
4weeks*Dec-2011	0.02 ± 0.01	1.75 ± 0.10^c	26.63 ± 3.20
2weeks*May-2012	0.02 ± 0.01	2.18 ± 0.21^b	30.82 ± 3.58
4weeks*May-2012	0.02 ± 0.01	5.43± 0.26^a	39.98 ± 3.47
Second growing season (2012-2013)			
Grazing frequency			
2 weeks	0.03 ± 0.02	2.70 ± 1.26	20.23± 9.67
4 weeks	0.06 ± 0.04	3.35 ± 1.31	19.52± 7.27
Date			
Oct-2012	0.01 ± 0.01	0.44± 0.09 ^{c}	0.55± 0.10 ^{c}
Jan-2013	0.09 ± 0.02	2.18 ± 0.53 ^{b}	20.03±3.80 ^{b}
May-2013	0.04 ± 0.04	6.87± 0.41 ^{a}	41.14±5.74 ^{a}

$^{a, b,c,}$ superscripts indicate values within columns that are significantly different (P<0.05)

[1] SEM, standard error of the mean

6.4.2 *In vitro* incubations

The nutritive analyses of plantain and chicory used as substrates are presented in Table 6-3. Chicory and plantain from plots harvested in May, 2013 had similar (P>0.05) CP and HWSC concentrations. Differences between plantain and chicory were found for ME, NDF, hemicellulose and OMD concentrations. NDF and hemicellulose concentrations were higher (P<0.05) in plantain compared to chicory whereas ME, OMD and the ratio of HWSC to structural carbohydrate (SC) were greater (P<0.05) in chicory compared to plantain.

Table 6-3 Nutritive analysis of plantain and chicory from the four weeks grazing frequency plots, harvested in May, 2013 and used as substrate (mean ± SEM[1]).

Nutritive value traits[2]	Plantain	Chicory
OM (g/kg DM)	856.6 ± 4.5	850.9 ± 5.4
Ash (g/kg DM)	143.4 ± 4.5	149.1 ± 5.4
CP (g/kg DM)	298.9 ± 11.9	322.5 ± 6.4
ME (MJ/kg DM)	12.15 ± 0.1 [b]	12.34 ± 0.4 [a]
NDF (g/kg DM)	188.7 ± 5.6 [a]	168.1 ± 5.2 [b]
ADF (g/kg DM)	148.3 ± 4.4	136.5 ± 5.6
Cellulose (g/kg DM)	74.5 ± 3.1	67.5 ± 16.5
Hemicellulose (g/kg DM)	40.3 ± 1.4 [a]	31.5 ± 0.9 [b]
Lignin (g/kg DM)	73.8 ± 2.8	69.0 ± 11.1
HWSC (g/kg DM)	85.5 ± 3.2	83.4 ± 6.1
OMD	0.74 ± 0.5 [b]	0.75 ± 2.7 [a]
Ratio (HWSC:SC)	0.75 ± 0.05[b]	0.91 ± 0.22[a]

[a, b] superscripts indicate values within rows that are significantly different (P<0.05)

[1]SEM, standard error of the mean

[2]DM, dry matter; OM, organic matter; CP, crude protein; ME, metabolizable energy; NDF, neutral fibre detergent; ADF, acid fibre detergent; HWSC, hot water soluble carbohydrates; OMD, organic matter digestibility; HWSC:SC, the ratio of HWSC to structural carbohydrates.

6.4.2.1 Gas production parameters after 24 h of incubation

The *in vitro* gas production parameters after 24 h of incubation are reported in Table 6-4. Chicory (CH) and plantain (PL) had similar (P>0.05) potential of GP but the total volume of gas produced after 24 h of incubation (V 24h) and the rate at which half the potential gas was produced ($R^{1/2A}$) were lower (P<0.05) for PL compared to CH.

The addition of acteoside to CH (CH+40ac) and PL (PL+36ac) increased (P<0.05) the potential of GP and the V24h of both than that of PL and CH alone. The addition of aucubin to CH (CH+10au; CH+20au) and PL (PL+10au) did not (P>0.05) impact the potential of GP and the V 24h compared to PL or CH. Although the addition of increasing concentrations of aucubin (10 and 20 mg/g DM) to the CH (CH+10au and CH+20au) caused a decrease (P<0.05) in the $R^{1/2A}$, the potential of GP and V 24h remain unchanged; aucubin may play a role in reducing the rate at which gas is produced in the rumen. This may perhaps partially explain the reduction of the potential of GP and V 24h observed for the PL *in vitro* fermentation compared to CH; however, the addition of extra aucubin to PL (PL+10au) did not result in reduction in GP.

Table 6-4 *In vitro* gas production parameters of the treatments after 24 h of incubation (mean ± SEM[1]).

In vitro Treatments[3]	Gas production parameters[2]			
	A (mL/g DM)	$T^{1/2A}$ (h)	$R^{1/2A}$ (mL/h)	V 24h (mL/g DM)
CH	221.4 ± 3.21[b]	4.4 ± 0.17[c]	21.1 ± 0.85[a]	220.3 ± 3.29[b]
CH+10au	219.1 ± 3.53[b]	4.6 ± 0.09[b]	20.2 ± 0.46[b]	217.9 ± 3.47[b]
CH+20au	215.1 ± 2.37[b]	5.0 ± 0.09[a]	18.3 ± 0.51[c]	213.3 ± 2.39[b]
CH+40ac	237.5 ± 5.90[a]	4.5 ± 0.10[c]	21.5 ± 0.48[a]	235.4 ± 5.16[a]
PL	210.9 ± 1.40[b]	4.7 ± 0.22[b]	17.0 ± 0.67[d]	207.5 ± 1.42[c]
PL+10au	214.2 ± 2.15[b]	4.4 ± 0.16[c]	18.1 ± 0.59[c]	211.1 ± 1.77[bc]
PL+36ac	235.7 ± 7.90[a]	4.5 ± 0.12[bc]	18.8 ± 0.59[c]	229.9 ± 5.82[ab]

[a, b] superscripts indicate values within columns that significantly differ ($P<0.05$). **[1]** SEM, standard error of the mean.

[2] Model parameters: A, potential gas production (mL/g DM); $T^{1/2A}$, time (h) at which half of A was reached; $R^{1/2A}$, rate (mL/h) at which $T^{1/2A}$ was reached; V24h, total gas produced at 24h (mL/g DM).

[3] *In vitro* treatments: CH, chicory; CH+10au, chicory+10 mg aucubin/g DM; CH+20au, chicory+20 mg aucubin/g DM; CH+40ac, chicory+40 mg acteoside/g DM; PL, plantain (containing endogenous levels of 7 mg aucubin/g DM and 36 mg acteoside/g DM); PL+10au, plantain+ extra 10 mg aucubin/g DM; PL+36ac, plantain+extra 36 mg acteoside/g DM.

6.4.2.2 End products after 24 h of incubation

The *in vitro* incubation pH and end products after 24 h of incubation are reported in Table 6-5. The CH incubation had a higher (P<0.05) pH and greater (P<0.05) total VFA and NH_3 concentration (Table 6-5) compared to the PL incubation after 24 h. In regards to individual VFA production after 24 h, the CH incubation produced proportionally more (P<0.05) acetate and branched chain VFA (BCVFA: isobutyrate and isovalerate), and less (P<0.05) propionate and butyrate compared to the PL incubation (Table 6-5). This resulted in the CH incubation having a greater (P<0.05) acetate to propionate (A:P) ratio than the PL incubation.

The addition of aucubin and acteoside to either CH or PL did not (P>0.05) alter pH, total VFA or NH_3 concentration produced by CH and PL after 24 h; however, the proportion of individual VFA was affected in the *in vitro* treatments after 24 h. The addition of acteoside to both chicory (CH+40ac) and plantain (PL+36ac) decreased (P<0.05) the proportion of acetate and increased (P<0.05) the proportion of propionate produced after 24 h, causing corresponding changes to the A:P ratio (Table 6-5). The addition of aucubin did not (P>0.05) alter the proportion of acetate or propionate produced by CH or PL after 24 h of incubation. However, the proportion of acetate was lower (P<0.05) when a level of 10 mg aucubin/g DM was added to chicory (CH+10au) compared to 20 mg aucubin/g DM (CH+20au).

The proportion of butyrate and BCVFA produced after 24 h of incubation was not affected (P>0.05) by adding aucubin or acteoside to either CH and PL. The addition of 10 mg aucubin /g DM to CH (CH+10au) did not affect (P>0.05) the proportion of BCVFA, but increasing the aucubin level at 20 mg/g DM in CH (CH+20au) a reduction (P<0.05) in the molar proportions of isobutyrate and isovalerate was observed in comparison to the pure CH incubation. Furthermore, the addition of acteoside to CH (CH+40ac) also resulted in a similar reduction (P<0.05) of isobutyrate and isovalerate in comparison to the pure CH incubation.

Table 6-5 ***In vitro*** **pH, total volatile fatty acid (VFA) concentration, molar proportion of individual VFA, ratio acetate to propionate (A:P), and ammonia (NH_3) concentration for chicory and plantain treatments after 24 h of incubation (mean ± SEM[1])**

In vitro treatments[2]	pH	Total VFA (mM)	Individual VFA (mmol/100 mL)					A:P	NH_3 mmol/g
			Acetate	Propionate	Butyrate	Isobutyrate	Isovalerate		
CH	6.48±0.01[a]	65.94±1.09[a]	68.15±0.22[ab]	17.66±0.20[d]	8.28±0.22[b]	1.78±0.03[a]	2.86±0.02[a]	3.86±0.04[a]	63.92±6.46[a]
CH+10au	6.48±0.01[a]	64.54± 1.94[a]	67.75±0.30[bc]	17.97±0.15[d]	8.38±0.28[ab]	1.80±0.04[a]	2.82±0.03[a]	3.77±0.02[b]	58.28±5.95[a]
CH+20au	6.48±0.01[a]	64.97± 1.19[a]	68.50 ±0.21[a]	17.52±0.15[d]	8.39±0.24[ab]	1.71±0.01[b]	2.66±0.06[b]	3.91±0.04[a]	56.43±3.56[a]
CH+40ac	6.47±0.01[a]	66.35± 0.86[a]	67.35± 0.20[c]	18.57±0.14[c]	8.12±0.21[b]	1.68±0.02[b]	2.65±0.03[b]	3.63±0.03[c]	64.43±4.03[a]
PL	6.43±0.01[b]	61.18±2.22[b]	67.28± 0.28[c]	19.01±0.15[b]	8.77±0.04[a]	1.34±0.02[c]	2.18±0.05[c]	3.54±0.03[d]	42.64±7.97[b]
PL+10au	6.42±0.01[b]	64.17±1.04[ab]	67.31± 0.19[c]	19.25±0.15[b]	8.66±0.06[a]	1.30±0.03[c]	2.11±0.07[c]	3.50±0.03[d]	34.14±2.21[b]
PL+36ac	6.41±0.01[b]	64.30±0.64[ab]	66.39± 0.31[d]	19.92±0.10[a]	8.53±0.07[a]	1.28±0.02[c]	2.07±0.06[c]	3.33±0.03[e]	37.72±3.40[b]

[a, b, c, d] Superscripts letters indicate values within columns that are significantly different ($P<0.05$). [1] SEM, standard error of the mean.

[2] *In vitro* treatments: CH, chicory; CH+10au, chicory+10 mg aucubin /g DM; CH+20au, chicory+20 mg aucubin/g DM; CH+40ac, chicory+40 mg acteoside/g DM; PL, plantain (containing endogenous levels of 7 mg aucubin/g DM and 36 mg acteoside/g DM); PL+10au, plantain+extra 10 mg aucubin/g DM; PL+36ac, plantain+extra 36 mg acteoside/g DM.

6.4.2.3 Volatile fatty acid production over time

Volatile fatty acids production over time (mmol/g DM) of acetate (Figure 6-1A), propionate (Figure 6-1B), total VFA (Figure 6-1C), and the BCVFA (isobutyrate and isovalerate) (Figure 6-2B) showed differences (P<0.05) between the *in vitro* treatments; however, butyrate (Figure 6-2A) production was similar (P>0.05) between the *in vitro* treatments.

Over 24 h of incubation, the CH incubation produced more (P<0.05) acetate, BCVFA, and total VFA, but similar (P>0.05) production of propionate and butyrate compared to the PL incubation. The addition of aucubin and acteoside to CH and PL affected acetate, propionate, and total VFA production (Figure 6-1); while butyrate and BCVFA production (Figure 6-2B) were not modified (P>0.05) by adding aucubin or acteoside to CH or PL.

Acetate production (Figure 6-1A) in CH and PL was not affected (P>0.05) by adding aucubin or acteoside to either CH (CH+10au; CH+20au; CH+40ac) or PL (PL+10au; PL+36ac) substrates. However, the addition of acteoside to both CH (CH+40ac) and PL (PL+36ac) increased (P<0.05) propionate production (Figure 6-1B) compared to all *in vitro* treatments. Nonetheless, the addition of acteoside to CH (CH+40ac) increased (P<0.05) the total VFA production (Figure 6-1C) by CH, but not in PL (PL+36ac).

Adding 20 mg/g DM of aucubin to chicory (CH+20au) produced less (P<0.05) acetate, propionate and total VFA compared to chicory with acteoside (CH+40ac) incubation. Moreover, the CH+20au treatment had an acetate and total VFA production similar (P>0.05) to plantain (PL; PL+10au; PL+36ac) treatments and a propionate production similar (P>0.05) to PL+10au and PL+36ac treatments.

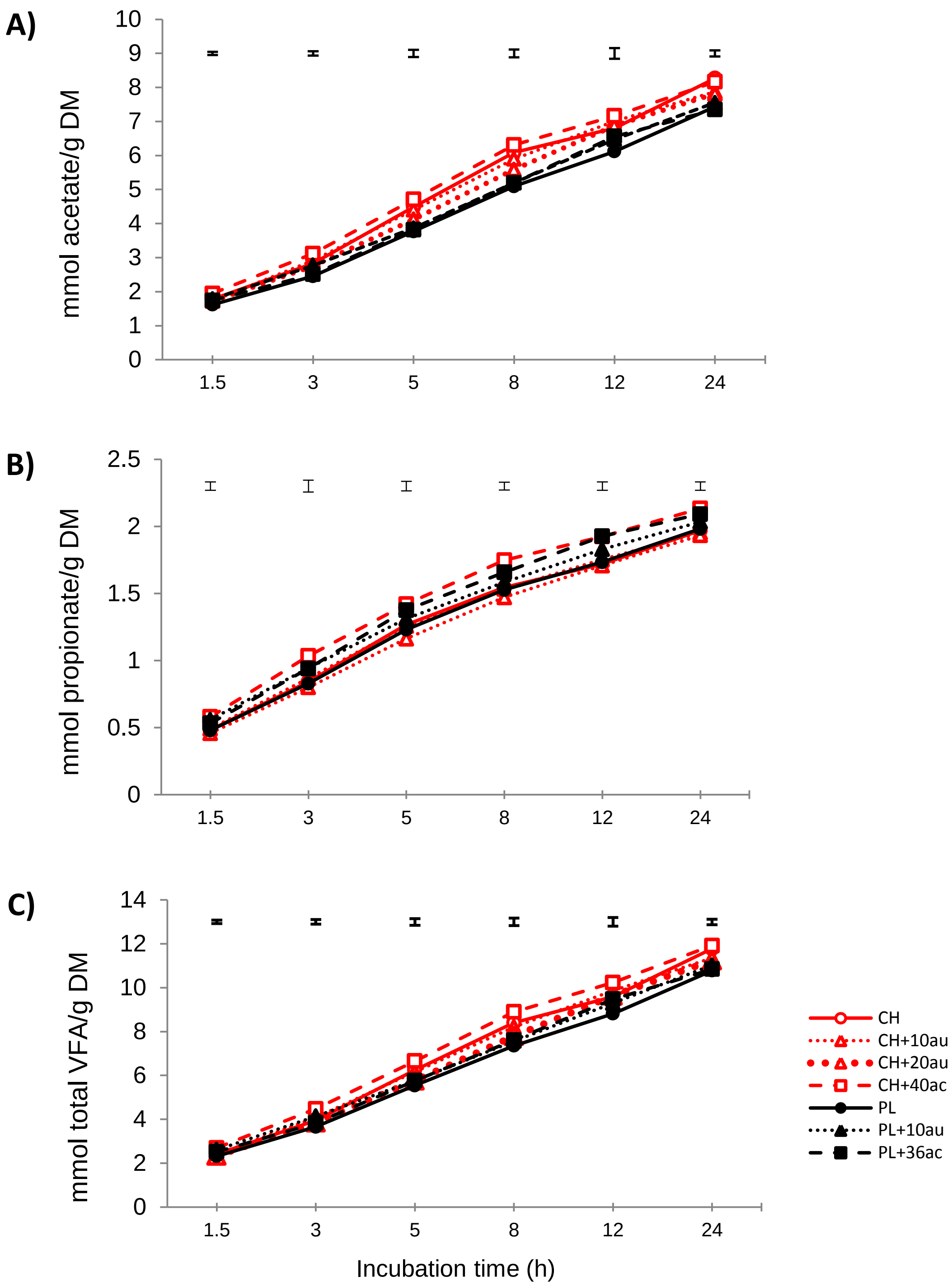
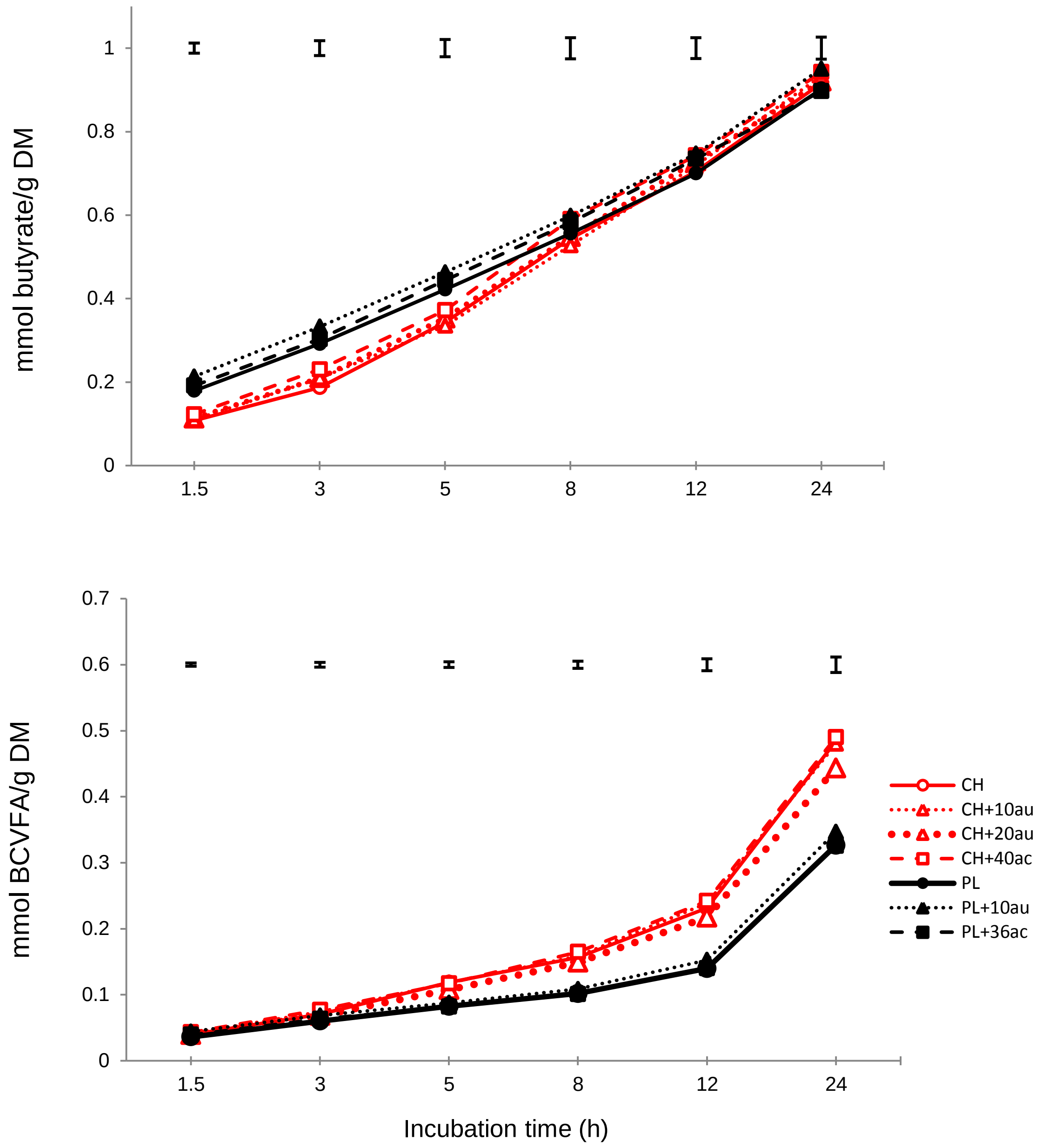


Figure 6-1 Production (mmol/g DM) over time of A) acetate, B) propionate, and C) total VFA in all the *in vitro* treatments: CH, chicory; CH+10au, chicory+10 mg aucubin/g DM; CH+20au, chicory+20 mg aucubin/g DM; CH+40ac, chicory+40 mg acteoside/g DM; PL, plantain (containing endogenous levels of 7 mg aucubin/g DM and 36 mg acteoside/g DM); PL+10au, plantain+extra 10 mg aucubin/g DM; PL+36ac, plantain+extra 36 mg acteoside/g DM. Bars denoting standard error of the mean (SEM) at each time point are included at the top of each figure.

A)



B)

Figure 6-2 Production (mmol/g DM) over time of A) butyrate and of B) branched VFA (isobutyrate and isovalerate) in all the *in vitro* treatments: CH, chicory; CH+10au, chicory+10 mg aucubin/g DM; CH+20au, chicory+20 mg aucubin/g DM; CH+40ac, chicory+40 mg acteoside/g DM; PL, plantain (containing endogenous levels of 7 mg aucubin/g DM and 36 mg acteoside/g DM); PL+10au, plantain+extra 10 mg aucubin/g DM; PL+36ac, plantain+extra 36 mg acteoside/g DM. Bars denoting standard error of the mean (SEM) at each time point are included at the top of each figure.

6.4.2.4 Net ammonia production over time

The net NH_3 production over time (mmol/g DM) increased ($P<0.05$) in plantain and chicory *in vitro* treatments (Figure 6-3). Over 24 h of incubation, plantain with the natural concentration of bioactive compounds (7 and 36 mg/g DM of aucubin and acteoside, respectively) produced 40% less NH_3 compared to chicory. During the first 5 h of incubation, there were no differences ($P>0.05$) in the net NH_3 production between plantain and chicory substrates, but after 8 h, plantain started to produce less ($P<0.05$) NH_3 compared to chicory.

The addition of aucubin (CH+10au; CH+20ac) and acteoside (CH+40ac) into chicory had the same impact, decreasing the net NH_3 production when compared to the CH incubation. However, it was found that the greater level of aucubin (20 mg/g DM) in chicory tended ($P=0.053$) to produce less NH_3 when compared to 10 mg aucubin/g DM in chicory (CH+10au). In plantain, the addition of aucubin (PL+10au) remained a similar ($P>0.05$) NH_3 production whereas the addition of acteoside in plantain (PL+36ac) decreased ($P<0.05$) the net NH_3 production when compared to PL.

The addition of 20 mg aucubin/g DM in chicory (CH+20au) had a stronger effect reducing the net NH_3 production when compared to chicory treatments (CH; CH+10au; CH+40ac) with a net NH_3 production similar ($P>0.05$) to PL and plantain with extra aucubin (PL+10au). However, plantain with extra acteoside (PL+36ac) was the treatment that most reduced the net NH_3 production over 24 h.

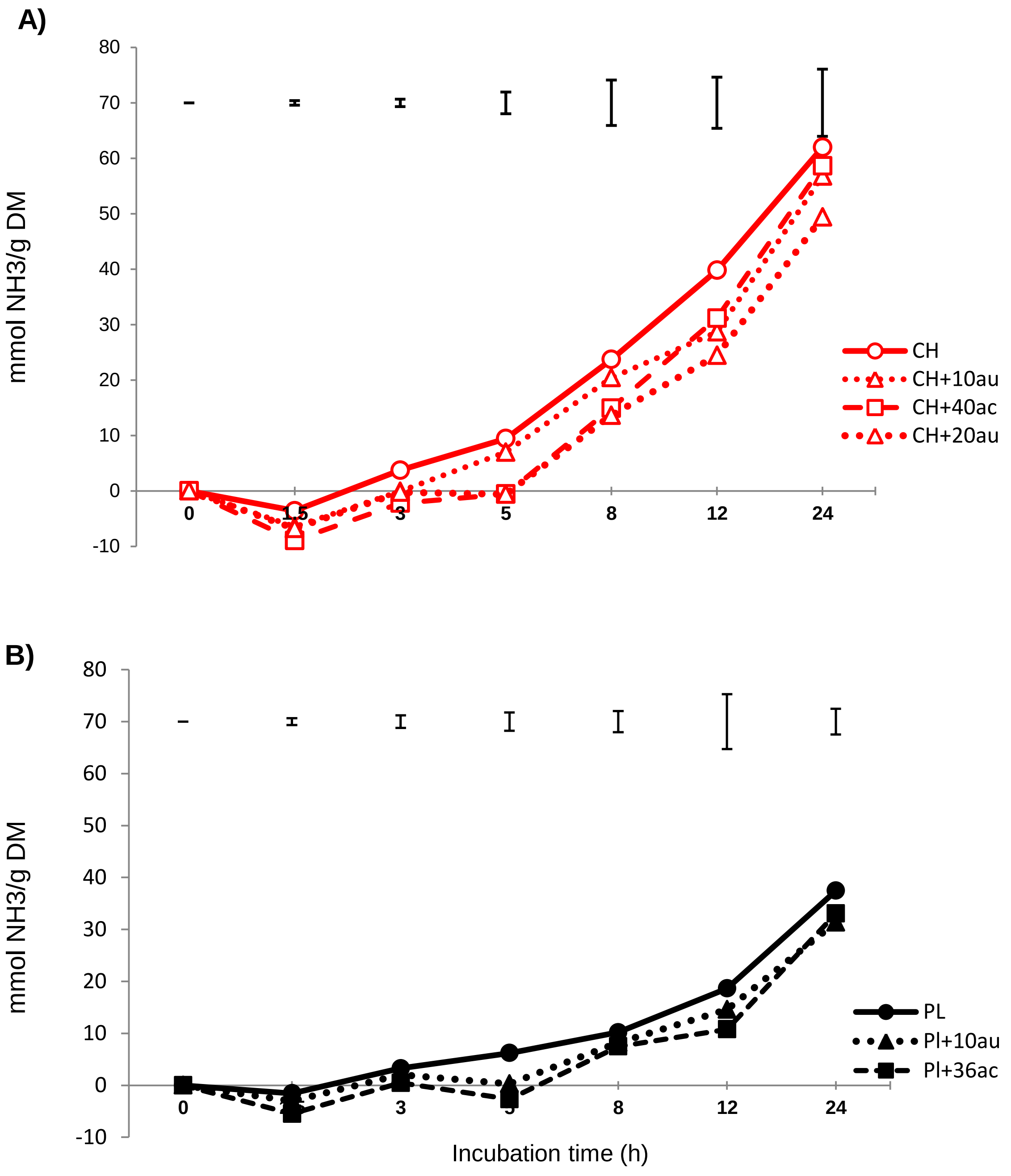


Figure 6-3 Net ammonia (mmol NH_3/g DM) production for A) chicory *in vitro* treatments: CH, chicory; CH+10au, chicory+10 mg aucubin/g DM; CH+20au, chicory+20 mg aucubin/g DM; CH+40ac, chicory+40 mg acteoside/g DM; PL, plantain (containing endogenous levels of 7 mg aucubin/g DM and 36 mg acteoside/g DM); PL+10au, plantain+extra 10 mg aucubin/g DM; PL+36ac, plantain+extra 36 mg acteoside/g DM. Bars denoting standard error of the mean (SEM) at each time point are included at the top of each figure.

6.5 DISCUSSION

6.5.1 Bioactive compounds in plantain.

Acteoside, aucubin, and very low levels of catalpol were detected in plantain cv. “Ceres Tonic” (Table 6-2). The concentration of these compounds in plantain is influenced by genetic and environmental factors (Fajer et al., 1992; Tamura and Nishibe, 2002), as well as leaf age (Rosenthal and Berenbaum, 1992). The very low levels of catalpol detected in plantain showed that in the cultivar ‘Ceres Tonic’ this iridoid glycoside is near absent ((Al-Mamun et al., 2008; Tamura, 2002)). Catalpol is biologically synthesised from its precursor aucubin (Damtoft, 1994). During the selection of the cultivar ‘Ceres Tonic’, the pathway from aucubin to catalpol appeared to be reduced to a very low basal rate of synthesis (Tamura and Nibishe, 2002). Catalpol can be found in all natural ecotypes of plantain and in the cultivar “Grassland Lancelot” (Tamura and Nibishe, 2002).

The concentrations of aucubin found in this study were lower than those reported earlier (Tamura and Nishibe, 2002). High concentrations of up to 30 mg aucubin/g DM depending on the genotype (Stewart, 1996), and from 10 to 27 mg aucubin /g DM for the cultivar “Ceres Tonic” have been reported (Tamura, 2002). However, acteoside can be present from 60 to 90 mg/g DM in natural ecotypes (Fajer et al., 1992) and from 15 to 41 mg/g DM in the cultivar ‘Ceres Tonic’ (Tamura and Nishibe, 2002), which are similar concentrations to those found in the present study. In contrast, Al-Mamun et al. (2008) reported concentrations as low as 3.2 mg/g DM of acteoside in the cultivar ‘Ceres Tonic’. The increase of aucubin and acteoside concentration in plantain swards throughout the growing season has been reported by others (Bowers and Stamp, 1992; Stewart, 1996; Tamura and Nishibe, 2002). The concentrations of aucubin and acteoside increased similarly in both growing seasons and the highest concentration of both compounds was in autumn (May, 2012 and May, 2013).

6.5.2 *In vitro* fermentation

In this study, plantain containing natural levels of aucubin and acteoside (7 and 36 mg/g DM, respectively) had the same potential for GP as chicory (Table 6-4). Similarly, fast ruminal degradation has been reported for both herbs with DM loss of 0.26 and 0.25 %/h for chicory and plantain, respectively (Burke et al., 2000). Differences in fermentation pattern are attributable to differences in the nutritive characteristics between substrates in the type of carbohydrate and its rate of depolymerisation (France and Dijkstra, 2005). The higher ME, OMD, HWSC:SC ratio and the lower NDF in chicory compared to plantain (Table 6-3) suggested chicory disintegrates more rapidly in the rumen than plantain (Barry, 1998). Also, plantain had slower rumen degradability compared to chicory due to its rate ($R_{1/2A}$) of GP which is directly proportional to the rate of degradation (France et al., 2005) and because after 24 h of incubation, the V 24h produced and the total VFA was lower in plantain than chicory (Table 6-4).

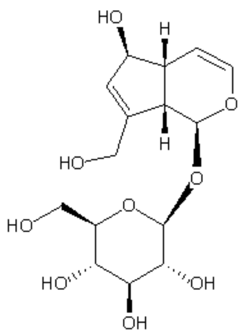
The higher total VFA concentration and production in chicory than in plantain (Table 6-5; Figure 6-1C) is agreement with (Burke et al., 2006). Volatile fatty acids are the end products of rumen fermentation and represent the main supply of ME for ruminants, a reduction in their production is considered nutritionally unfavourable for animal production (Burke et al., 2000; Busquet et al., 2006). The feeding value (FV) for chicory has been always consistently superior than plantain for ruminants (Barry, 1998; Hoskin et al., 1995; Komolong, 1994; Kusmartono et al., 1996). Plantain produced a lower total VFA and acetate production than chicory after 24 h of incubation (Figure 6-1). Similarly, *in vivo* experiments with deer have shown a trend for total VFA and acetate to be less when deer were fed plantain compared to perennial ryegrass (Swainson and Hoskin, 2006). Although, the higher NDF and hemicellulose content in plantain compared to chicory might encourage the growth of acetate producing bacteria (France and Dijkstra, 2005), the higher pectin levels reported for chicory

(Barry, 1998; Jackson et al., 1996; Sun et al., 2011) likely contributed to the production of more acetate (Marounek and Duskova, 1999).

Acteoside caused an increase in GP, propionate, and hence in the total VFA compared to aucubin. The increase in GP with acteoside reflects an increase in the fermentable substrate. Propionate is produced via the succinate: propionate pathway by some ruminal bacterial and CO_2 is formed increasing GP (Lila et al., 2003). Both acteoside and aucubin are glycosides with antimicrobial properties (Kim et al., 2000; Tamura and Nishibe, 2002). However, rumen microbes might be either able to tolerate its effect or be capable of degrading due to containing non-specific glycosidase that might break the compound down (Getachew et al., 2002). Acteoside is a phenylpropanoid (with caffeic acid) and phenylethanoid (with hydroxytyrosol) glycoside (Figure 6-4B). Acteoside always has glucose as the central sugar, but rhamnose, the other carbohydrate, may be replaced or be present as additional auxiliaries (Rønsted et al., 2000). In general, compounds with phenolic structures are considered to have stronger antimicrobial activity in comparison with other non-phenolic secondary plant compounds due to the presence of a hydroxyl group in the phenolic structure (Helander et al., 1998; Ultee et al., 2002). Both gram-positive (most acetate- and butyrate-producing bacteria) and gram-negative bacteria (normally propionate-producing bacteria) have been inhibited by carvacrol (phenolic terpenoid) in a dose-dependent manner (Van Nevel and Demeyer, 1977). However, this study suggests that the sugar components from acteoside were used as an energy source to produce propionate both in chicory and plantain.

Conversely, aucubin is an iridoid glycoside with an O-linked glucose at C-1 (Figure 6-4A); the sugar can be hydrolysed by *B*-glucosidase forming the aglycone of aucubin, aucubigin (Berenbaum and Rosenthal, 1992). The aucubin aglycone has been identified as affecting bacteria and fungi (Davini et al., 1986). Therefore, the opposing effect between CH+20au and CH+40ac suggests that aucubin resulted in a general inhibition of rumen microbial fermentation due to the negative effect on acetate, propionate, and total VFA production. It may be possible that aucubin negatively affected acetate-producing bacteria, showing its bactericide effect. The changes in individual VFA are most likely to changes in the microbial community in the rumen (Wina et al., 2005), as rumen microbial activity is affected by plant secondary metabolites (Busquet et al., 2006). Thus, differences in fermentation products could be attributed to the presence of the bioactive compounds, aucubin and acteoside, in plantain. The presence of aucubin in plantain seems to be responsible of the lower VFA and acetate production in plantain, while acteoside improved propionate production to levels similar to chicory.

A)



B)

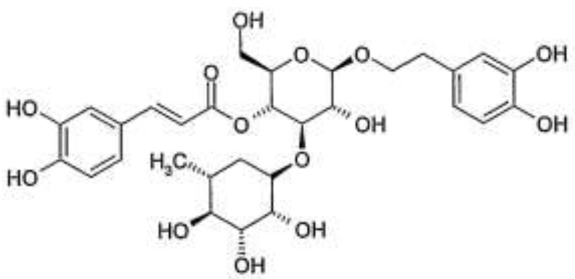


Figure 6-4 Chemical structure of A) aucubin and B) acteoside

6.5.3 Ammonia production

This study showed that chicory produced more NH_3 than plantain. Ammonia utilisation by microbes in the rumen is primarily carbohydrate-limited, and increasing carbohydrate availability in the rumen improves the efficiency of microbial utilisation of NH_3 (Russel et al., 1991). Chicory has shown to reduce the NH_3 concentration in the rumen when compared to cocksfoot (*Dactylis glomerata* L.) (Komolong, 1994), and perennial ryegrass (Sun et al., 2012), due to its higher ratio of RFC:SC providing an increased energy supply for microbial growth (Barry, 1998). The ammonia N disappearance with increased energy availability has been higher due to its incorporation into microbial protein (Raab et al., 1983). However, the higher ratio of RFC:SC in chicory should suggest a lower NH_3 production than in plantain. The reduced NH_3 production after 24 h of incubation, consistent with the net ammonia (NH_3/g DM) produced over time in plantain might be due to secondary compounds. In this regard, Swainson and Hoskin (2006) have speculated that the markedly reduced NH_3 concentration in the rumen fluid of deer fed plantain compare to those fed pasture to be due to the presence of secondary compounds in plantain. Additionally, a lower N concentration in the urine was obtained with cows grazing diverse pasture which included plantain, but it was not determined if the effect was by improvement of N efficiency or N dilution in the urine (Totty et al., 2013).

The net NH_3 production indicates the magnitude at which the protein degradation exceeds the capacity for microbial utilisation and the likelihood that plant N is insufficient for microbial growth (Chaves et al., 2006). Since the majority of rumen bacteria use NH_3 as their N source, the availability of NH_3 is important for microbial protein production (Satter and Slyter, 1974), and the microbial yield of VFA may be restricted by NH_3 insufficiency (Raab et al., 1983). However, NH_3 concentration was always above the minimum requirement (5 mg/dL) for microbial growth in these incubations (Satter and Slyter, 1974).

Both aucubin and acteoside reduced the net NH_3 production in both chicory and plantain demonstrating the potential effect of both compounds for reducing urinary N excretion and minimising N losses to the environment. Several mechanisms have been suggested for how secondary compounds can reduce NH_3 concentration in the rumen, such as: (i) reduction in peptidolysis and deamination; (ii) direct inhibition of rumen microbial growth; or (iii) inhibition of rumen hyper-ammonia producing bacteria (Durmic and Blache, 2012). The observed reduction in the molar proportion of BCVFA in chicory with aucubin (20 mg/g) and acteoside (40 mg/g) suggests that both compounds reduce amino acid deamination. A reduction in NH_3 by deamination is associated with a reduction of the BCVFA (isobutyrate and isovalerate) (Chalupa et al., 1980; Horton et al., 1980). The BCVFA are derived from amino acid catabolism in the rumen (Mackie and White, 1990). An inhibition of amino acid deamination has practical implications because it may increase ruminal escape of dietary protein and improve the efficiency of N use as has been shown with thymol (Broderick and Balthrop, 1979). However, as the BCVFA production over time was not affected by the addition of aucubin and acteoside to any substrate, the changes in the proportion molar of the BCVFA are likely a consequence of the greater total VFA production in chicory with acteoside (CH+40ac) and the lower total VFA production in chicory with 20 mg/g of aucubin (CH+20au).

The reduced NH_3 concentration with aucubin in this study was unlikely to be a consequence of an increased utilisation for microbial synthesis, due to the antimicrobial properties of these compounds (Tamura and Nishibe, 2002). Aucubin aglycone has been identified as affecting bacteria and fungi by binding to free amino acid making them unavailable and contributing to its biological and toxic effect (Davini et al., 1986; Kim et al., 2000). The fact that aucubin added to chicory (20 mg/g) had the same effect as plantain in decreasing NH_3 production may be a consequence of the bactericide effect of this compound. In contrast, although acteoside has also been described as antimicrobial (Andary et al., 1982) and antifungal (Shoyama et al., 1986), the mechanism of its action is not clear. Consequently, an antimicrobial action by acteoside in this study appears less likely than a reduction of NH_3 due to greater fermentation resulting from acteoside. Consequently, acteoside seemed to reduce the NH_3 production due to an increase in the efficiency of N utilisation in ruminants. The presence of acteoside in plantain capable of affecting the rumen fermentation process is likely to have important implications for rumen N efficiency (Stewart, 1996), and suggests that selecting plantain for higher acteoside concentration would have positive animal effects by improving the overall VFA profile and reducing N losses in the urine.

6.6 CONCLUSIONS

Chicory forage, which is absent of aucubin and acteoside, produced more NH_3, acetate and total VFA compared to plantain forage containing natural levels of both compounds. Both acteoside and aucubin present in plantain reduced the NH_3 production and affected the VFA profile. Acteoside improved rumen fermentation by increasing propionate production and total VFA, while aucubin reduced acetate and total VFA production. This antagonist effect between aucubin and acteoside suggested that aucubin had a bactericide effect on ruminal microbes while acteoside was most likely utilised as an energy source for microbial growth. In conclusion, acteoside in plantain would have the potential to reduce the N losses in the urine and improve the overall VFA profile with positive effects on animals.

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7 OVERALL DISCUSSION AND CONCLUSIONS

7.1 OVERALL THESIS OBJECTIVE

Chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) are being used by dairy farmers in New Zealand to supplement dairy cows during summer/autumn, when herbage production and quality of perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) pastures decline. Substantial research has evaluated chicory and grazing management recommendations with sheep and deer (Clark et al., 1990; Li and Kemp, 2005; Powell et al., 2007); however, there have been many anecdotal cases of poor chicory persistence on dairy farms with current grazing management practices. Moreover, there is still limited information available for plantain and its grazing management on dairy farms (Kemp et al., 2014; Lee et al., 2015).

This thesis reports a series of experiments conducted in the field and laboratory to evaluate the production, persistence, plant development, feed quality, and animal selection of three pastures, chicory, plantain, and a herb-clover mix under two different grazing frequencies. These results were presented and discussed in detail in chapters 3 to 6. In brief, chapter 3 evaluated the herbage production, persistence, and plant development of chicory and plantain in pure and in herb-clover mix pastures; chapter 4 compared the nutritive value of these pastures; chapter 5 determined the diet selection of the herbs and herb-clover mix by dairy cows; and chapter 6 determined the content of bioactive compounds, aucubin and acteoside in plantain and its effect on *in vitro* rumen fermentation. This overall discussion chapter presents an integrated discussion of the key findings in the experimental chapters and will focus on pasture persistence, plant development, and feed quality supply of the pastures, and the effect of the bioactive compounds on *in vitro* rumen fermentation.

7.2 PRODUCTION AND PERSISTENCE OF HERB PASTURES

Chapter 3 demonstrated that plantain and the herb-clover mix were more productive during the two growing seasons evaluated than chicory and perennial ryegrass/white clover pasture. Plantain and the herb-clover mix produced approximately 33% and 21% more herbage during the first (2011-2012) and second (2012-2013) growing seasons, respectively, than chicory (Table 3-3). Both chicory and plantain sown as pure swards or in herb-clover mix pasture showed an initial high plant density; however, plantain retained a greater plant density than chicory in both the pure swards and in the herb-clover mix pasture (Figure 3-3). A greater herbage production and plant density for plantain compared to chicory has been reported in previous studies conducted in New Zealand (Glassey et al., 2013; Lee et al., 2015; Powell et al., 2007). The greater plant density of plantain in the herb-clover mix likely explained the higher DM production of this pasture compared to chicory; this is due to the close relationship between herbage production and plant density (Neal et al., 2009).

This thesis highlights that grazing frequency affected the plant density of chicory but not for plantain. Grazing chicory every two weeks was unfavourable for the persistence of chicory, causing a greater loss of plant density compared to grazing every four weeks (Lee et al., 2015; Li and Kemp, 2005). This work demonstrated that grazing chicory every two weeks caused the chicory swards failed to persist from January of the second growing season (<10 plants/m^2; <25 shoots/m^2) and at this time, more than half of the herbage production in these swards came from white clover and weeds (Figure 3-2A). Consequently, this pasture would not persist for a second summer when feed deficit is expected and supplementation becomes essential in dairy farming. Chicory grazed every four weeks retained a greater plant density and shoot density with a higher herbage mass contribution from chicory for the two growing seasons evaluated (Figure 3-5).

In contrast, plantain in both the pure swards and in the herb-clover mix maintained a similar plant density regardless of whether grazing every two or every four weeks. Although the optimal plant density for high yielding plantain pastures has not yet been established, the plantain plant density obtained at the end of the grazing trial (approximately 170 plants/m^2) was greater than the optimal plant density recommended for chicory pastures (>50 plants/m^2) to ensure high herbage production (Li and Kemp, 2005). Consequently, these results showed that plantain and the herb-clover mix were more productive and persisted better than chicory irrespective of grazing frequency during the two growing seasons.

7.2.1 Plant development

This thesis highlights that chicory and plantain plants respond differently to grazing frequency. Both herbs are taproot species and store carbohydrate reserves in their roots as an energy source for winter survival, growth initiation in spring, and for regrowth after defoliation (Kemp et al., 2010; Li et al., 1997a). However, chicory plants had greater taproot diameter than plantain (Powell et al., 2007; Sanderson and Elwinger, 2000), and, therefore, stored a higher concentration of carbohydrate reserves in their roots compared to plantain (Table 3-5).

Grazing frequency affected root development in chicory but not in plantain. Chicory plants grazed every two weeks did not develop large taproots compared to those grazed every four weeks and, thus, stored less carbohydrate reserves (fructan and fructose) in their roots for regrowth after grazing. Chicory plants were unable to replenish carbohydrate stores with only two weeks between grazing, and failed to develop extra shoots. As result, grazing chicory every two weeks may kill plants by depleting their carbohydrate reserves compared to chicory grazed every four weeks. Chicory grazed every four weeks not only had an increased number of shoots per plant, especially from late spring to early summer, but also maintained a higher shoot density and greater herbage mass contribution from chicory in the swards

(Figure 3-5), which compensated the decline in plant density (Li et al., 1997b; Teixeira et al., 2007).

Plantain plants retained similar taproot diameter regardless of grazing frequency. Smaller roots and a lower concentration of root reserves in plantain compared to chicory may suggest that plantain regrowth after grazing does not depend on root carbohydrate reserves to the same extent as chicory. Plantain plants responded to frequent grazing (every two weeks) by increasing their number of shoots per plant compared to those grazed every four weeks. Plantain appeared to utilise its root reserves for shoot growth to maintain a leaf area for photosynthesis; hence, it might be more dependent on residual leaf area than root reserve carbohydrates.

7.3 FEED QUALITY SUPPLY

The herbage produced from the three pastures had a high nutritive value for milking cows during summer and autumn. These pastures were characterised in Chapter 4 with a high metabolisable energy (ME) content (> 11 MJ/kg DM), low neutral detergent fibre (NDF) concentration (<350 g NDF/kg DM), and a crude protein (CP) concentration unlikely to be limiting for milk production (Hodgson and Brookes, 1999; NRC, 2001). The three pastures presented a ME content numerically higher than perennial ryegrass/white clover pasture during summer (Chapter 4; Figure 4-1B) when perennial ryegrass/white clover is generally at its lowest ME content (Waghorn and Clark, 2004).

Furthermore, the weather conditions during the first (2011-2012) growing season were in contrast to the second (2012-2013) growing season (Chapter 3). During the first growing season, summer was wet and perennial ryegrass increased its ME content rapidly from summer to autumn. However, in the second growing season, spring and summer were notably dry, and perennial ryegrass/white clover pasture showed a ME content lower in summer through to autumn than the herb pastures (Chapter 4; Figure 4-1B). These results demonstrate that the low summer ME content of perennial ryegrass/white clover pasture would be pronounced under drought conditions through autumn (Lee et al., 2013), whilst the herb and herb-clover mix pastures continued to provide a high quality feed supply until autumn. Consequently, the three pastures evaluated could be considered a good option to complement perennial ryegrass/white clover pastures to alleviate the decline in feed quality and lower animal production during summer and autumn.

Although the annual dry matter (DM) production of the herbs and the herb-clover mix pastures did not differ when grazing every two or every four weeks (Chapter 3; Table 3-3), grazing every two weeks reduced the proportion of chicory and plantain in the pure swards compared to those grazed every four weeks. While, the contribution of the sown species in the herb-clover mix were more stable under both grazing frequencies. The DM contribution from the sown species in the swards is important in terms of maintaining feed quality supply (Tozer et al., 2011). There was a greater DM contribution from chicory and plantain when grazed every four weeks, and the herb-clover mix best performed due to a greater herbage production and higher DM contribution of the sown species under both grazing frequencies.

The herbs and herb-clover mix pastures produced enough additional high-quality feed during summer/autumn to supplement dairy cows. The greater herbage mass when the pastures were grazed every four weeks suggests that these pastures might allow greater stocking rate, meaning from 400 to 450 cows/ha at herbage allowance of 10 kg DM/cow/day from December to April, compared to those grazed every two weeks (Chapter 3; Figure 3-1A). The herbage intake of grazing animals has been reported to be greater at higher herbage mass than at low herbage mass (Forbes and Hodgson, 1985; Orr et al., 2004). Although grazing frequently (every two weeks) improved the nutritive value of the herbs and herb-clover mix by increasing CP and decreasing NDF concentrations compared to those grazed every four weeks (as previously alluded in Chapter 4), dairy cows avoided the pastures grazed every two.

Dairy cows showed greater grazing preference for the herb-clover mix than for pure swards of chicory or plantain (Chapter 5; Figure 5-6), which demonstrated that dairy cows preferred a mixed swards rather than a pure sward (Parsons et al., 1991; Rutter et al., 2004). Dairy cows grazing the herb-clover mix selected indiscriminately between chicory, plantain and red clover during late spring and summer regardless of grazing frequency (Chapter 5; Figure 5-3), indicating a high palatability and high intake potential for this pasture (Barry, 1998; Stewart, 1996). This finding combined with the high herbage mass suggests higher DM intakes (DMI) by dairy cows grazing the herb-clover mix (Chapter 5; Figure 5-6).

Chicory and plantain grazing preference varied between seasons. This seasonal preference was affected by the availability and accessibility of herbage and its acceptance by the animal, as reported by others (Baumont et al., 2000; Dumont et al., 2002; Ungar and Noy-Meir, 1988). The increased diet selection from late spring to summer of plantain, as a pure sward and in the herb-clover mix, are likely related to a lower stem contribution in the swards during summer compared with late spring. Palatability problems in plantain have been associated with stem development (Fraser and Rowarth, 1996; Swainson and Hoskin, 2006). The greater stem production during late spring affected the overall nutritive value of plantain. Nevertheless, the better flexibility of plantain and the herb-clover mix in regards to grazing frequency suggest that farmers could graze these pastures more frequently to improve their overall nutritive value (Chapter 4).

7.3.1 Effect of plantain bioactive compounds on *in vitro* fermentation

Chapter 6 demonstrated that aucubin and acteoside, bioactive compounds found in plantain, reduced the net ammonia (NH_3) production demonstrating the potential effect of both compounds for reducing urinary nitrogen (N) excretion and minimising N losses to the environment. Both acteoside and aucubin showed an antagonist effect on the volatile fatty acid (VFA) profile. Aucubin reduced acetate and total VFA production, suggesting a bactericide effect on ruminal microbes (Bartholomaeus and Ahokas, 1995; Davini et al., 1986; Kim et al., 2000). However, acteoside appeared to improve rumen fermentation by increasing propionate and total VFA production and was likely utilised as an energy source for microbial growth. Consequently, acteoside appeared to reduce NH_3 production due to an increase in the efficiency of N utilisation associated with greater microbial activity. This suggests that selecting plantain for higher acteoside concentration may have positive animal effects by improving the overall VFA profile and reducing the N losses in the urine.

7.4 LIMITATIONS OF THESIS STUDIES

There are some limitations to consider in the studies reported in this thesis. These include:

1. In Chapter 3 and 4, the perennial ryegrass/white clover plots were not assigned randomly in the experimental design. Therefore, the perennial ryegrass/white clover pasture was not included in the statistical analysis to compare with the pasture treatments. The values of annual DM production and nutritive value reported for the perennial ryegrass/white clover pasture in this work represent a numerical benchmark and statistical comparisons between the pasture treatments and perennial ryegrass/white clover pasture cannot be made.

2. In Chapter 5, the diet selection of dairy cows was examined in the herb-clover mix as affected by the accessibility (horizontal and vertical) of the species in the swards; and the grazing preference for the herbs and herb-clover mix pastures when grazing every two or every four weeks. However, the chemical characteristics of these species and pasture treatments were not analysed at the same time as the diet selection. Hence, it was not possible to state and relate the physical and chemical characteristics of the pastures on the diet selection of dairy cows.

3. In Chapter 6, only the higher concentrations of the bioactive compounds were examined to investigate their impact on *in vitro* rumen fermentation. This was due to the limited capacity of the incubator available (32 bottles) and the cost of the purified compounds.

7.5 FURTHER RESEARCH

This work provided evidence in relation to the effect of grazing frequency on the production, persistence, plant development and feed quality of chicory and plantain as pure swards and a herb-clover mix pasture managed with dairy cows throughout two growing seasons. Chicory and plantain showed different strategies to persist under grazing. Chicory grazed at four week intervals by dairy cows persisted for the two growing seasons, but was likely to fail in a third growing season. Chicory has been shown to persist for at least three growing seasons when grazed by sheep and deer; however, chicory grazed by dairy cows may require different management. Further research on chicory should consider grazing frequencies longer than four weeks and perhaps grazing only until the end of March to improve root development and carbohydrate reserves for winter survival.

Plantain retained a similar plant density and taproot diameter regardless of grazing frequency, persisting better than chicory. This suggests the plantain did not utilise its carbohydrate reserves for regrowth to the same extent as chicory, but rather depended on residual leaf area for regrowth. Plantain grazed every two weeks responded by increasing its number of shoots per plant in order to maintain leaf area. Therefore, further research on plantain should evaluate the effect of residual height post grazing on the production and persistence of this pasture species.

The effect of the bioactive compounds, aucubin and acteoside, present in plantain demonstrated a reduction in the NH_3 production and to alter the VFA profiles from *in vitro* rumen fermentation. Further research on these compounds is needed to confirm that they cause the same effects in animals using *in vivo* experiments. Additionally, future studies would be needed to evaluate the amount of urine produced, the N concentration in that urine and the effect of these compounds on nutrients digestibility.

7.6 MAIN CONCLUSIONS

This thesis demonstrated that:

1. Grazing every two weeks was detrimental for the persistence of chicory, whereby chicory grazed by dairy cows every two weeks failed to persiste for two consecutive growing seasons. However, chicory grazed by dairy cows every four to a residual height of between 7-10 cm persisted for the two growing seasons.

2. Grazing frequency did not affect plant density and root development in plantain. Plantain and the herb-clover mix were more productive and persisted for the two growing seasons irrespective of grazing frequency and, hence, may be easier to manage under grazing than chicory.

3. The three pasture options produced sufficient herbage of high-quality to supplement dairy cows during summer/autumn. However, dairy cows preferred the herb-clover mix, selecting chicory, plantain and red clover indiscriminately regardless of grazing frequency, rather than pure chicory or plantain pastures.

4. The bioactive compounds, aucubin and acteoside, present in plantain reduced NH_3 production. Aucubin reduced acetate and total VFA production likely due to its bactericide activity, while acteoside increased propionate and total VFA suggesting improved rumen fermentation.

This research showed that both plantain and the herb-clover mix appeared to be more durable under the two grazing managements and persisted better than chicory. Combined, the results suggest that herb-clover mix pastures may be more suitable for use in dairy systems with environmental advantages. They have the ability to provide large quantities of high-quality feed during the summer/autumn period, are resilient to grazing frequency, and are highly preferred by dairy cows. This pasture had the potential to reduce N excreted in the cow urine due to the bioactive compounds present in plantain. These results provide sufficient evidence to recommend the use of herb-clover mix pastures in dairy farm systems.

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