The release of nitrogen and carbohydrate from herbage during comminution.

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In forage-based livestock production systems, dietary N is often in excess supply. In addition, the efficiency with which ruminants utilise dietary N utilisation is low, leading to substantial losses of N to the environment, particularly in urine. There are considerable political and societal pressures for farmers to reduce N losses, yet still maintain productivity. To enable reductions in N excretion and improve N utilisation, the flow of N through ruminants needs to be understood. While much is known about the ruminal degradation of dietary N, less is known about the release of N from herbage during ingestion. As herbage N is readily fermentable, the extent of release of N during ingestion has implications for ruminal ammonia concentration and concomitantly, N excretion. The aims of this PhD research programme were to quantify the variation between forages in N release during comminution of herbage, explore the characteristics of herbage that influence N release, and consider implications for N excretion. Two laboratory based experiments were conducted to examine the release of N during mechanical maceration of herbage, as a proxy for the effect of ingestive mastication on herbage by dairy cows. The first compared N release from herbage of five commonly used forages: perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.), lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), and plantain (*Plantago lanceolata* L.), collected in February, May, August, and November of 2015. The amount of N (as crude protein, CP) released during maceration of herbage varied 15-fold, with lucerne releasing the most (11.5 g/100 g DM) and plantain the least (0.8 g/100 g DM). Crude protein release from forages ranked as follows: lucerne > ryegrass = clover > chicory > plantain. Investigations revealed that no single characteristic (i.e. herbage physical properties or chemical composition), measured in this study, was strongly associated with CP release of all forages evaluated.

However, forages that tended to have greater herbage CP and non-protein N (NPN) concentrations tended to release more N during maceration. This finding led to the second laboratory experiment that explored the effect of increasing rates of N fertiliser application (0, 100, 200 and 350 kg N/ha/y) on herbage characteristics and release of N. Herbage was collected from swards of perennial ryegrass, lucerne, chicory and plantain in May (autumn) and November (spring) 2015. Increasing N fertiliser rate applied increased leaf length (up to 140%), herbage mass (up to 320%), and CP and NPN concentration in perennial ryegrass, chicory and plantain. Increasing N fertiliser rate doubled the amount of CP released from ryegrass herbage across both seasons, and chicory in autumn, but increased CP release from plantain 6-fold in autumn. Increasing N fertiliser rate applied to ryegrass, chicory and plantain swards also reduced the ratio of fermentable carbohydrate to CP in herbage, which is thought to increase loss of N in urine. The greatest increase in the amount of CP released and greatest decline in the ratio was observed at N rates exceeding 200 kg N/ha/y, coinciding with reductions in herbage mass and leaf growth response to increased rates N fertiliser application. This study suggests rates equivalent to 200 kg N/ha/y ought to be the upper limit of N fertiliser applied to swards of ryegrass, chicory and plantain.

The third experiment conducted sought to determine the effect of ingestive mastication by mature dairy cows on fresh cut herbage from three forages (perennial ryegrass, lucerne and chicory). The ranking of herbages for CP release was similar to that determined in the first two laboratory based experiments, where 7.5, 5 and 3 g CP/100 g DM was released from lucerne, perennial ryegrass and chicory, respectively, during ingestive mastication. Release of CP was associated with the concentration of CP in herbage ($R^2 = 0.53$) supporting the findings of the previous laboratory experiments. When N intake rate was calculated, delivery of N to the rumen was greatest in cows fed ryegrass (0.79 g N/min) compared with lucerne and chicory (0.54 and 0.22 g N/min), which would have implications for microbial utilisation in the rumen. Despite the slower intake rate of chicory compared to lucerne and ryegrass (46.5 vs. 64.7 and 70.7 g DM/min) the extent of comminution was less in chicory compared to the other forages (36 vs 44 and 41% of particles reduced to < 4 mm), but the difference was not associated with any of the herbage characteristics measured in this study, nor was comminution shown to be associated with CP release when data from the three forages were combined for analysis.

This research programme concluded with a simulation study exploring milk-solids (MS) production and urinary N excretion from cows grazing swards of perennial ryegrass, lucerne, chicory and plantain grown under increasing rates of N fertiliser (0, 100, 200, 350 and 500 kg N/ha/y) using the data collected in the second experiment. Predictions of MS production and N excretion by the MINDY model for cows grazing chicory, ryegrass or plantain, suggests that rates of N fertiliser of 200 kg/ha/y could be the optimal point for MS production before total daily N excretion increases substantially. As at this application rate, DMI and MS production predicted for cows grazing chicory was near maximum (15.9 kg DM/cow/d; 1.24 kg MS/cow/d) with little gain achieved through additional N fertiliser but a 100 g N/cow/d increase in urine N excretion (42% increase) was predicted as N applied increased from 200 – 500 kg N/ha/y. In the case of plantain and ryegrass, while MS production was predicted to increase beyond the 200 kg N/ha/y fertiliser rate, the amount of N excreted also increased by 65 and 103 g N/cow/d, respectively (50 and 64%). The simulations also suggest that the addition of N fertiliser to lucerne swards has little effect on MS but increases urine N excretion, and is thus not recommended.

Overall, the findings of this research program confirm the hypothesis that the release of N from herbage during comminution differs between forages. This finding can aid explanation of literature that has demonstrated differences in the N excretion by cows fed diets of different forages, and can help understanding of N flow in ruminants. The study also suggests that in autumn, there is potential to manipulate N release from perennial ryegrass, chicory and plantain herbage during maceration and to reduce N excretion from cows grazing diets of these forages through altering the amount of N applied to these swards. This information could be used to inform fertiliser management practices, and development of diets for dairy cows to reduce urinary N loss.

Keywords: biomechanical, cell rupture, chemical composition, chicory, comminution, dairy cow, digestion, forage, ingestive mastication, lucerne, maceration, modelling, nitrogen excretion, nitrogen utilisation, nutrient release, plantain, rumen ammonia, ryegrass, urinary nitrogen, white clover.

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List of Abbreviations

Abbreviation	Description
°C	Degrees Celsius
A	Soluble fraction in an *in sacco* digestibility study
a.i.	Active ingredient
ADF	Acid detergent fibre
ANOVA	Analysis of variance
B	Degradable insoluble fraction in an *in sacco* digestibility study
Bw	Bolus wet weight
BW	Body weight
c	Cow
CC	Cell contents
CHO	Carbohydrate
CP	Crude protein
CPS	Soluble crude protein
CV	Coefficient of variation
d	Day
DM	Dry matter
DMY	Dry matter yield
DW	Dry weight
F	Force
FV	Feed value
FW	Fresh weight
g	Gram
h	hour
ha	Hectare
J	Joule
k	Fractional degradation rate in an *in sacco* digestibility study
kg	Kilogram
km	kilometre
L	Litre
LSD	Least significant difference
m	Meter
m^2	Meter square
ME	Metabolisable energy
MF	Maximum force applied (Newtons)
min	Minute
mm	Millimetres
MPS	Microbial protein synthesis
N	Nitrogen
n	Number
NDF	Neutral detergent fibre
NFC	Non fibre carbohydrate
NH_3	Ammonia

Abbreviation	Description
NDIN	Neutral detergent insoluble nitrogen
NIRS	Near infrared spectroscopy
NPN	Non-protein nitrogen
NSC	Non-structural carbohydrate
NUE	Nutrient use efficiency
NV	Nutritive value
P	Potentially degradable fraction in an *in sacco* digestibility study
PSD	Particle size distribution
RDP	Rumen degradable protein
s	Second (time)
SA	Sum of amps
SC	Structural carbohydrate
SD	Standard deviation
SE	Standard error
SED	Standard error of the difference
SEM	Standard error of the mean
TMR	Total mixed ration
TP	True protein
U	Undegraded fraction in an *in sacco* digestibility study
UDP	Undegradable crude protein
V	Volt
VFA	Volatile fatty acids
WSC	Water soluble carbohydrate
WW	Wet weight
y	Year

1

Introduction

Dairy farming in New Zealand is based on the rotational grazing of pasture swards, commonly comprised of a binary mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). This is because the climate and physical environment of the main dairying regions allow for growth of these species for most of the year (Valentine and Kemp, 2007), providing a relatively low cost feed system (Dillon et al., 2005). Swards are typically grazed in a vegetative state in order to maximise herbage yield and nutritive value, but this also results in high (> 18%) herbage crude protein (CP; nitrogen × 6.25) concentration (Ulyatt, 1997), and consumption of dietary CP in excess of livestock requirements (Kolver, 2000; NRC, 2001). Furthermore, dairy cows poorly utilise dietary nitrogen (N). Studies investigating the amount of dietary N used for milk production equates to only 20 – 30% of N consumed when fed ryegrass (Van Vuuren et al., 1993; Astigarraga et al., 1994; Peyraud et al., 1997). Any dietary N not utilised is excreted in the urine and faeces, at a metabolic cost to that animal, and is a source of environmental pollution (Scholefield et al., 1993; Pacheco and Waghorn, 2008).

The environmental issue involves scale, as New Zealand currently has about 6.6 million dairy cows (StatisticsNZ, 2016). One dairy cow consuming 15 kg DM per day, containing 18% CP in mid-lactation will consume 430 g of nitrogen (N) and excrete approximately 160 g N/day in the urine; that same cow consuming more, higher CP containing herbage (18 kg DM at 29% CP) will excrete approximately 520 g N/day in the urine (Waghorn et al., 2007). Generally, as the amount of N excreted increases, the concentration of N in urine increases. Then depending on the concentration of N in urine, the volume urinated and the area the urine is spread over, the N loading rate in a urine patch can be very high. In a review of the literature, Selbie (2014) determined that the rate of N loading can range between 200 – 2000 kg N/ha, and the main factor influencing this is urine N concentration, which in turn, is largely driven by dietary CP intake. High loading rates can exceed the capacity of what forage plants can uptake, and it is the excess

N not used by plants that is at risk of leaching to groundwater. Therefore, the number of animals and the CP concentration of their diet influence N leaching risk.

Currently, there is considerable social pressure to improve the quality of New Zealand's freshwater resources. Regional councils are obliged to establish targets for fresh water quality under the 2014 National Policy Statement for Freshwater Management that must be implemented by 2025 (NZGovernment, 2014). However, the New Zealand dairy industry is worth $14 billion/year, contributing 36% to revenue from primary industries (StatisticsNZ, 2017). Ruminants also play a vital role in global food supply by converting feed that is inedible to humans into high value protein for human consumption, the demand for which is increasing (Dijkstra et al., 2011; Lee et al., 2015c). A compromise is needed between maintaining or improving productivity and profitability while minimizing environmental impact. It is for this reason, that agricultural industry strategies implemented in the past decade have included focus on sustainable production, and research is required to help achieve this.

Options for reducing N excretion per cow include lowering the CP content of the diet and improving the efficiency of N use for livestock production (i.e. conversion of dietary N to N in milk or tissue). Reducing CP content in the diet of grazing cows may be achieved through the use of forage species that contain lower concentrations of CP in herbage compared with ryegrass/clover mixtures, or potentially, through managing the N fertiliser applied to swards. Increasing N fertiliser application to ryegrass swards increases ryegrass herbage CP concentration (Goswami and Willcox, 1969), but there is less information about how N fertiliser application affects the CP concentration, and the forms that make up that CP (i.e. NPN, CP fractions), of other common forage species (i.e. chicory, *Cichorium intybus*; and plantain, *Plantago lanceolata*). Similarly, NUE can theoretically be improved by feeding species that have more optimally balanced CP and water-soluble carbohydrate (WSC) concentrations in their herbage (Phuong et al., 2013). Parsons et al. (2011) suggested that reduction in urinary N excretion is observed when the WSC to CP ratio exceeds 0.7. While the chemical composition of ryegrass has been widely researched, exploration of the chemical composition of other forage species, and the potential to manipulate the composition to improve N utilisation is required.

Livestock nutrient use efficiency (NUE) may also be influenced by the rate and extent of nutrient supply to the rumen. Several studies have described the ruminal degradation of feeds (Burke et al., 2000; Burke, 2004; Chaves et al., 2006; Hammond et al., 2014), but less is known about nutrient release in the preceding step, that is, during ingestion. Ingestion is the first step in feed degradation and involves prehension of the herbage, then comminution by mastication and manipulation of the herbage into a bolus that can be swallowed. Waghorn et al. (1989) et al. suggested between 50 – 70% of cells are ruptured during ingestion. In grazed forages, most nitrogenous compounds and all soluble carbohydrates are contained within the plant cell walls, and are available to the rumen microflora once cells are ruptured. Therefore, the extent and pattern of cell rupture influences delivery of nutrients to the rumen pool. Boudon et al. (2001, 2006) investigated the release of nutrients from perennial ryegrass by ingestive mastication of dairy cows and determined that different nutrients were more readily released than others. While Acosta et al. (2007) demonstrated that the degree of comminution and the amount of nutrient released from perennial ryegrass, tall fescue and white clover during ingestion by dairy cows, differed between forages ($P < 0.05$). Expanding upon this research to include other forages would improve our understanding of nutrient utilisation. Neither Acosta et al. (2007) or Boudon et al. (2001, 2006) were able to conclusively determine what particular characteristics of the herbage influenced degree of communion or nutrient release. Pond et al. (1984a), when investigating the variation in extent of comminution between Coastal bermudagrass (*Cynodon dactylon*) and perennial ryegrass stated that: "There is a need for considering the animals influence as well as the structural influence of various forages in order to achieve a more complete understanding of the forage x livestock interaction." In addition, there is no known researching investigating whether N fertiliser management could influence nutrient (primarily, N) release during ingestion. Therefore, the aim of this study was to define the physical and chemical characteristics of common forage species, and relate these to the extent of comminution and release of nutrients. The effect of rate of N fertiliser application to swards on herbage characteristics will be explored. This understanding is required to determine whether efficiencies in nutrient use can be improved, and if nitrogen excretion can be reduced by forage species selection and N fertiliser management.

1.1 Research objectives

1. To quantify the variation in nutrient release from herbage of five common forage species when macerated, and define the characteristics of herbage that influence nutrient release.
2. To quantify the effect of nitrogen fertiliser application rate on herbage characteristics, comminution and release of nutrients.
3. To evaluate the effect of ingestive mastication on herbage from forage species which are physically and chemically contrasting.
4. To integrate data into a mechanistic simulation model to predict feeding behaviour, nitrogen metabolism, and partitioning of N from dairy cows fed diets of different forage species grown under a range of N fertiliser application rates.

1.2 Hypotheses

1. H_0: The extent of herbage nutrient release differs between species.
2. H_0: Relationships exist between herbage characteristics and the extent of comminution and nutrient release during maceration.
3. H_0: The extent of release of nutrients from herbage during maceration can be manipulated through N fertiliser management.
4. H_0: Predictions of dairy cow N metabolism and excretion will vary when grazing species grown under variable N fertiliser management regimes.

1.3 Thesis structure

This thesis consists of 8 Chapters. Chapter 2 reviews the literature with particular reference to the characteristics of forage species that influence herbage degradation and N metabolism in the dairy cow. Chapter 3 details the experimental site and management of the field plots from which herbage material was obtained. The development of methods for assessing the biomechanical properties is also outlined in Chapter 3. The following Chapter (Chapter 4) aims to quantify the variation in nutrient release from herbage of five common forage species during maceration and

seeks to define the characteristics of herbage that influence nutrient release and comminution of herbage during mechanical maceration in the laboratory. In Chapter 5, the relationship between nutrient release and the application of nitrogen fertiliser management is explored. Chapter 6 investigates the effects of ingestion by dairy cows on herbage comminution and nutrient release. The final experimental Chapter (Chapter 7) uses data collected in Chapter 4 in a modelling simulation exercise to predict the feeding behaviour and nitrogen metabolism of dairy cows fed herbage grown under a range of N fertiliser application rates. A diagrammatic representation of this thesis structure is given in Figure 1.1.

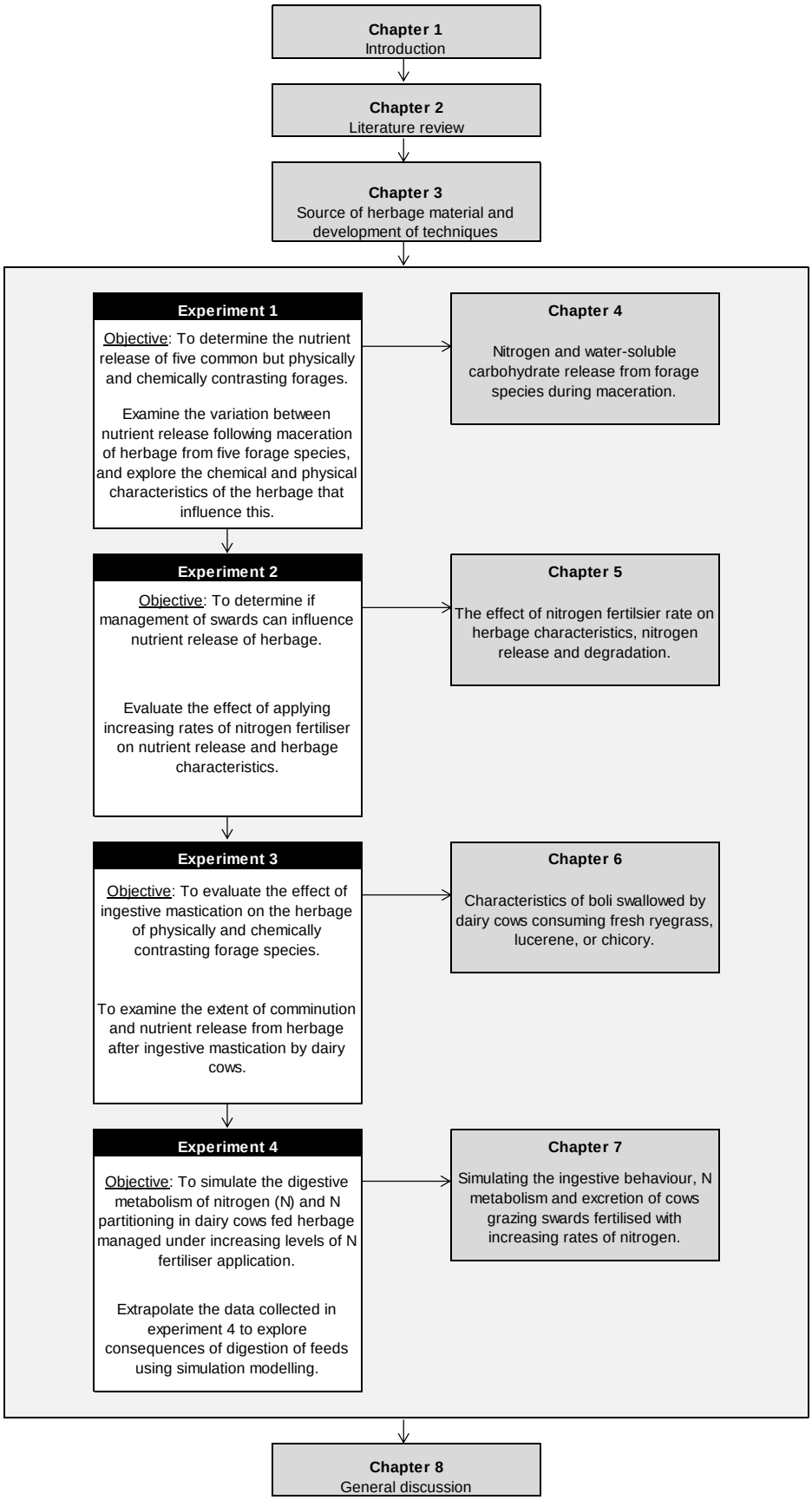


Figure 1.1. Diagrammatic representation of thesis structure.

2

Review of the literature

This review is concerned with the characteristics of forage species that influence how herbage from these is degraded during ingestion and ruminal digestion by dairy cows in a pastoral grazing environment. Published research on forage physical characteristics and herbage chemical composition, with relevance to digestion and utilisation of nutrients is summarised and discussed. Of particular interest are herbage characteristics that influence degradation and nutrient release during the initial phases of digestion (ingestion and ruminal degradation), and to what extent this can be manipulated through sward management. Understanding these parameters may allow greater understanding of, and improvements in nutrient use efficiencies in relation to diet and indicate opportunities for reduction of nitrogen excretion.

2.1 Pastoral grazing and the environment

Grazed swards provide the bulk of the feed on dairy farms in New Zealand. The dominant sward type is based on perennial ryegrass (*Lolium perenne* L.), commonly sown in mixture with white clover (*Trifolium repens* L.). Perennial ryegrass (ryegrass)-based swards are extensively used because they are easy to establish, can produce large amounts of good quality feed and are tolerant of grazing (Kemp et al., 2000b). In order to maximise production (i.e. dry matter and milk-solids yield) from ryegrass-based swards, they are grazed during the vegetative growth phase and commonly grown with the addition of nitrogen (N) fertiliser to promote growth. These management practices tend to produce herbage with high concentrations of N (Ulyatt, 1997; Lambert et al., 2004). Herbage with high concentrations of N (3.2 g/100g DM; 20% CP) can mean that dietary N supplied to the animal exceeds their physiological demand for maintenance and production (Tamminga, 1992b). Excess dietary N is excreted. Nitrogen metabolism and the efficiency of N utilisation by ruminants is discussed further in sections 2.4 and 2.5.

In New Zealand, N loss from agricultural systems has been identified as a main contributor of environmental pollution. Since the 1990's dairying has intensified, by increasing stock numbers

(from 3.8 million cows in 1994 to 6.6 million in 2016), stocking rate (cows/ha), and by the amount of N fertiliser used (WRC, 2015; StatisticsNZ, 2016; 2017). Trends in the N loading to land from agricultural systems indicate that the contribution from dairy has been increasing (Figure 2.1.) (Scarsbrook and Melland, 2015). Over a similar period, monitoring of fresh water bodies in New Zealand has shown a trend for declining water quality (i.e. elevated nutrient loading and eutrophication) (Verburg et al., 2010; Ballantine and Davies-Colley, 2014). Consequently, it has become a priority for the New Zealand government to improve the management of freshwater resources, and has done so by compiling a National Policy Statement for Freshwater Management that requires all regional councils to develop objectives for water quality which must be implemented by 2025 (NZGovernment, 2014). Alongside this, the dairy industry has also deemed research into the reduction of N loss a high priority (DairyNZ, 2014b).

In order to understand how N loss may be minimised, the flow of N through agricultural systems must be understood.

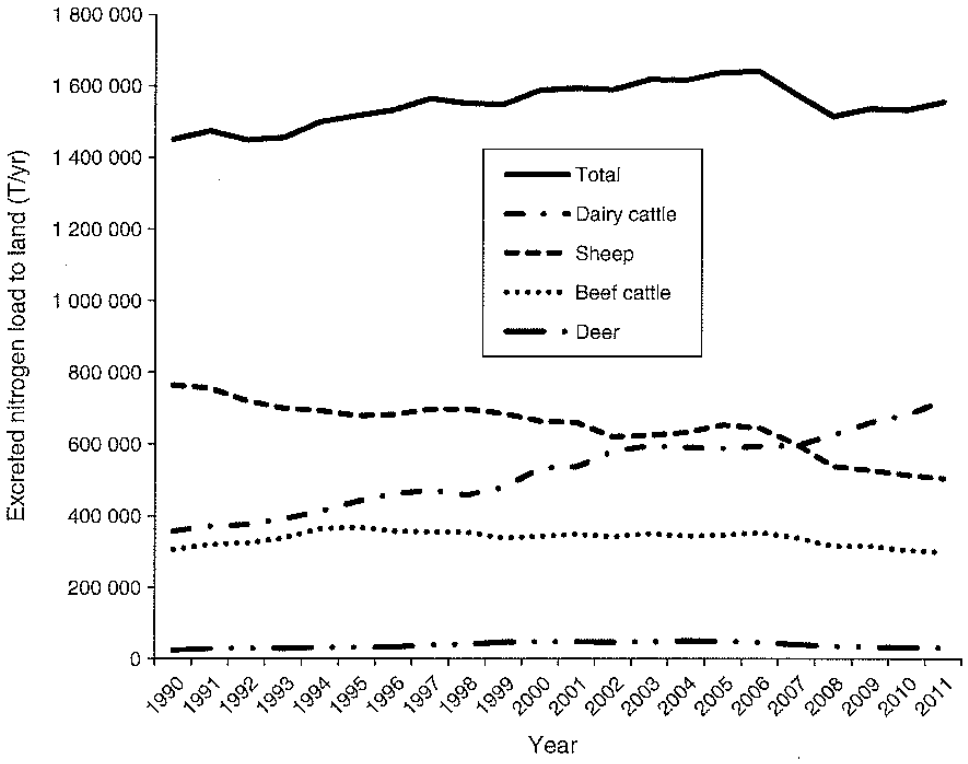


Figure 2.1. National trends in nitrogen load excreted to land (t/y) from all stock types for the period 1990 – 2011 in New Zealand. Sourced with permission: Scarsbrook and Melland (2015).

2.2 The nitrogen cycle

Figure 2.2 illustrates the generalised flow of N in agricultural systems based on grazing livestock. Briefly, N enters the N cycle in soil through decomposition of organic matter, deposits of excreta from livestock and by the application of fertilisers. These N inputs are converted to ammonia (NH_3) then ammonium (NH_4^+) by the process of ammonification. Because ammonification is a biological process, the rate will vary depending on soil temperature, moisture and aeration. Ammonium ions may be absorbed by plants, fixed to clay or undergo nitrification by microorganisms and converted to nitrate (NO_3^-). Nitrate is the most plant available form of N, but because it is negatively charged like clay particles, it is not adsorbed by the soil. Therefore, any nitrate that is not taken up by plants is susceptible to loss from the soil via runoff or leaching. The rate of nitrate leaching depends on plant uptake, soil drainage and rainfall. Nitrate that is not leached or used by plants may undergo denitrification and be released to the atmosphere as gas which can also be a source of environmental pollution (Whitehead, 1995; Johnson et al., 2005).

Efficient N management occurs when there is adequate N available to plants during periods of active growth (and thus N uptake), and minimising excess N that will lead to losses to the environment. The main source of nitrate leaching under grazing systems is that from livestock urine, and to a lesser extent directly from N fertiliser application (Ledgard et al., 1999b). Consequently, reducing the amount of N lost via excretion has become a focus of research (Woodward et al., 2012; Bryant et al., 2017).

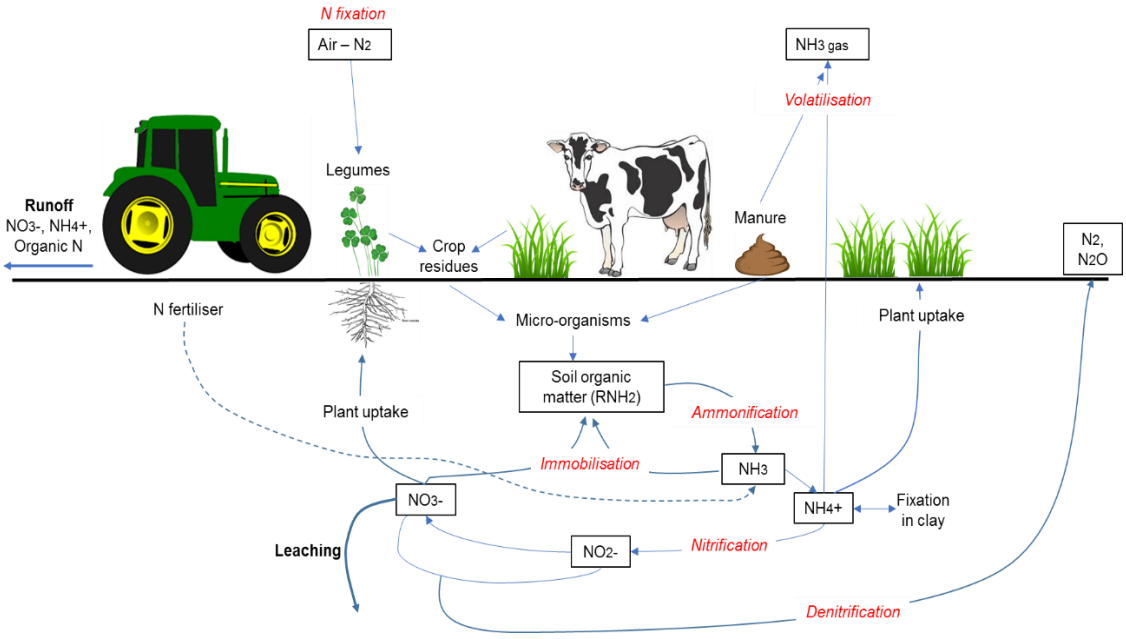


Figure 2.2 A generalised depiction of the nitrogen cycle within livestock grazing systems. (Adapted from: Tamminga, 1992; Johnson et al., 2005).

2.3 Digestion of feed by dairy cows

For the purpose of this review, a forage plant is defined as a plant that is grown to be utilised by grazing livestock *in situ* (adapted from definitions by Givens (2000) and Fageria (1997)). In New Zealand, common forage species fall into the categories such as: grasses, herbaceous legumes and herbs (Stewart et al., 2014).

Dairy cows are ruminants, as such, their gastrointestinal tract is characterised by a four-compartment stomach, including the rumen (or reticulo-rumen) from which the animals regurgitate and re-chew their food (rumination) (Figure 2.3). The degradation of feed by ruminants involves two consecutive stages: ingestive mastication and ruminal degradation.

2.3.1 Ingestive mastication

Mastication (i.e. chewing) is the integral first step of the digestion of feed. Structural damage to the plants outer layer (epidermis) by mastication allows microbial access to inner tissues that would otherwise only be accessible via the stomata (Cheng et al., 1980; Wilson, 1993). While ruminants can regurgitate and re-chew feed, ingestive mastication plays a significant role in reducing herbage fragment size and increasing surface area of herbage material for microbial colonisation and degradation (Ulyatt et al., 1986; Noziere et al., 2010). The reduction in herbage

fragment size also contributes to the formation of a bolus that is able to be swallowed. Waghorn et al. (1989) showed that mastication reduced 61% of lucerne and 46% of ryegrass to a size able to pass a 2 mm sieve; while Lee and Pearce (1984) demonstrated that up to 50% of dry feed (straw or hay) was reduced to fragment sizes less than 1 mm during mastication. These studies indicate that there is considerable variation in the effect of ingestive mastication on herbage between forages or feed types (i.e. fresh vs. conserved). The characteristics of herbage contributing to this variation and influences on subsequent degradation and utilisation of nutrients, however, are less well understood.

A further function of mastication is the rupture of plant cells and release of nutrients that can then be fermented by microbes. Waghorn and Clark (2004) reported that between 50 – 80% of herbage cells are ruptured during mastication. Since a large proportion of plant readily fermentable nutrients (i.e. N and carbohydrate, CHO) are encapsulated within the plant cell walls, the extent and pattern of cell rupture by mastication impacts the supply of nutrients to the rumen. Which in turn, effects rate and extent of rumen fermentation and the utilisation of nutrients (Sauvant and Van Milgen, 1995). Published data on nutrient release from herbage by mastication ranges from 22 – 86% depending on the both the species and the nutrient (Reid et al., 1962; Waghorn and Shelton, 1988; Boudon et al., 2002). Boudon and Peyraud (2001) showed that 60% of fermentable carbohydrate were released during mastication, but only 30% of chlorophyll (where much of the intracellular N is contained) was released, largely owing to the larger size of the molecule. Again, the variation in nutrient release between plant species is large and the reasons for this not well understood. Improving our knowledge of how different species are degraded during mastication would improve our understanding of digestion.

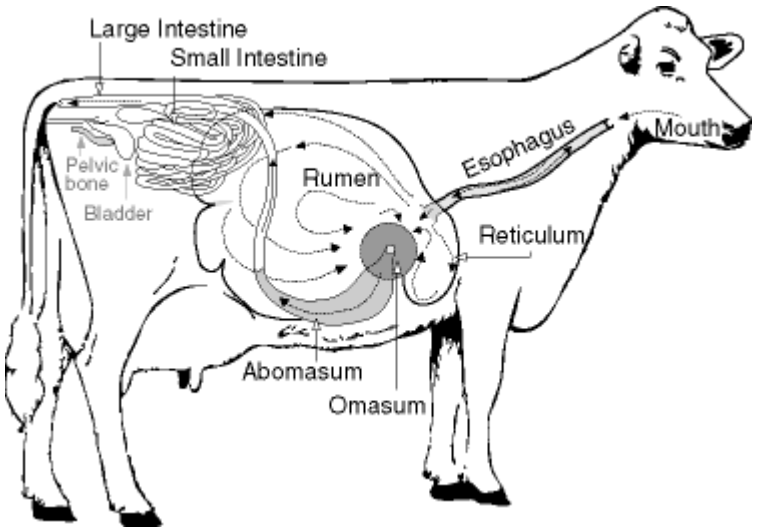


Figure 2.3 Digestive system of the cow. Sourced with permission: Wattiaux and Howard (1999)

2.3.2 Ruminal degradation and digestion

The rumen is anaerobic and inhabited by a complex and diverse population of symbiotic microorganisms (bacteria, archaea, protozoa and fungi) that degrade plant structural fibre and protein that the cow would otherwise be unable to digest by its own enzymes (Czerkawksi, 1986). Feeding the cow is to feed the microorganisms, as it is the end products of microbial digestion (volatile fatty acids (VFA) and amino acids), lipids and the microbes themselves that provide nutrition to the cow. For example, VFA production from microbes provides 70% of the energy absorbed by the cow (Waghorn and Clark, 2004), and bacteria are responsible for the degradation of much of the dietary protein into forms useable by the cow (section 2.4).

Nutrients are broken down by microbes in the rumen by the combined processes of solubilisation and degradation. Solubilisation is the release of nutrients from plant cells during ingestion and rumination. Solubilised nutrients, including proteins and fermentable CHO, are almost immediately degraded, with rates of 300 and 200%/hr respectively reported (Mangan, 1982; Weisbjerg et al., 1998). Degradable proteins are also released via cell rupture but are degraded more slowly, 4 – 47%/hr (Min et al., 2000). A rapid degradation of protein following mastication could result in excessive amounts of ammonia accumulating in the rumen (Attwood et al., 1998) which has implications for nitrogen use efficiency and excretion (section 2.4 and 2.5).

The degradation phase occurs when swallowed particles enter the rumen, and are mixed with digesta by a series of contractions, bringing the newly ingested herbage into contact with the microbiota. The rumen microbial population consists of species of bacteria, protozoa, fungi and archaea, and may be either free in the rumen liquid or attached to feed particles. The plant cell wall matrix is degraded by adherent microbes secreting enzymes which reduce the carbohydrates into monomers (by hydrolysis) which are then transported into the microbial cell for their own metabolism, generating end products such as VFAs, that the host animal can use (Nagaraja, 2016).

Further particle size reduction occurs with rumination (Poppi et al., 1980; Noziere et al., 2010) and to a small extent by microbial degradation (Murphy and Nicoletti, 1984; Wilson et al., 1989a; Wilson et al., 1989b). Particles remain in the rumen for varying lengths of time depending on their degradation rate and the passage rate of material out of the rumen (~ 20 – 48 hours for fibre particles). To enable passage of particles out of the bovine rumen, generally particles must be reduced to a size able to pass a sieve with a 2.0 mm aperture (Waghorn and Clark, 2004). Thus the rate that particles are degraded influences their retention time of the particles in the rumen and the rate of nutrient release from the herbage. This in turn, affects how much of the *potentially* degradable nutrients are degraded i.e. particles may flow from the rumen before they have been fully degraded (Gill et al., 1966).

Particles that have been reduced to a size able to pass from the rumen, flow with the liquid into the omasum and to the fourth compartment, the abomasum. The abomasum is similar to monogastric stomachs where acids and enzymes are secreted to continue the digestive process. Further absorption and digestion of protein, lipids and fibre occurs in the small intestine and in the hind gut where further fermentation and water absorption takes place, after which undigested material is excreted.

2.4 Nitrogen metabolism in the ruminant

The pathways of N metabolism are illustrated in Figure 2.4. Dietary protein can be divided into that which is potentially degradable in the rumen (RDP) and that which cannot be degraded (rumen undegradable protein, RUP). Rumen degradable protein can be further divided into true

protein (TP) N or non-protein N (NPN). Non-protein N consists of N present in DNA and RNA, amides, amines, free amino acids, urea and nitrate (Tamminga, 1986). Degradation of dietary N involves catabolism of protein by microbial proteases into peptides and amino acids, some of which are eventually deaminated into ammonia N which can be used for microbial growth (provided there is a supply of energy) and become incorporated into microbial protein (McNabb et al., 1996; Bach et al., 2005) i.e. the protein in rumen microflora. If the ammonia is not used for microbial protein synthesis (MPS), it will be absorbed into the bloodstream. Consequently, the concentration and rate of ammonia production in the rumen can reflect both the amount of soluble and the degree of degradable N in the diet (Huntington and Archibeque, 2000). Ammonia absorbed is processed into urea in the liver and excreted in the urine, at an energetic cost to the animal of 30 kJ ME/g N (Tyrrell et al., 1970; Waghorn et al., 2007). If dietary N is insufficient, however (< 1.2 g N/100 g DM; Minson (1991)), urea can be recycled via saliva back into the rumen to support microbial growth for several weeks.

Forage species generally contain high amounts of potentially degradable protein (rumen degradable protein, RDP) (> 70%), and degradation of N in the rumen is typically extensive (Beever and Siddons, 1984; Van Vuuren et al., 1992). Microbial protein synthesis may contribute 40 – 90%, of the amino acids supplied to the small intestines (Storm and Orskov, 1983). Once in the small intestine, protein is hydrolysed into small peptides and amino acids and absorbed for use by the animal. Remaining N is excreted in the faeces, consisting mainly of microbial debris (Mason, 1969; Nolan, 1996). Amino acids are used in the mammary gland for milk production, tissues and the excess are degraded in the liver and N is excreted as urea in the urine (Wattiaux, 1998; Pacheco and Waghorn, 2008). Overall, rumen microbes can use any form of N, but the cow requires several essential amino acids that are derived from the microflora.

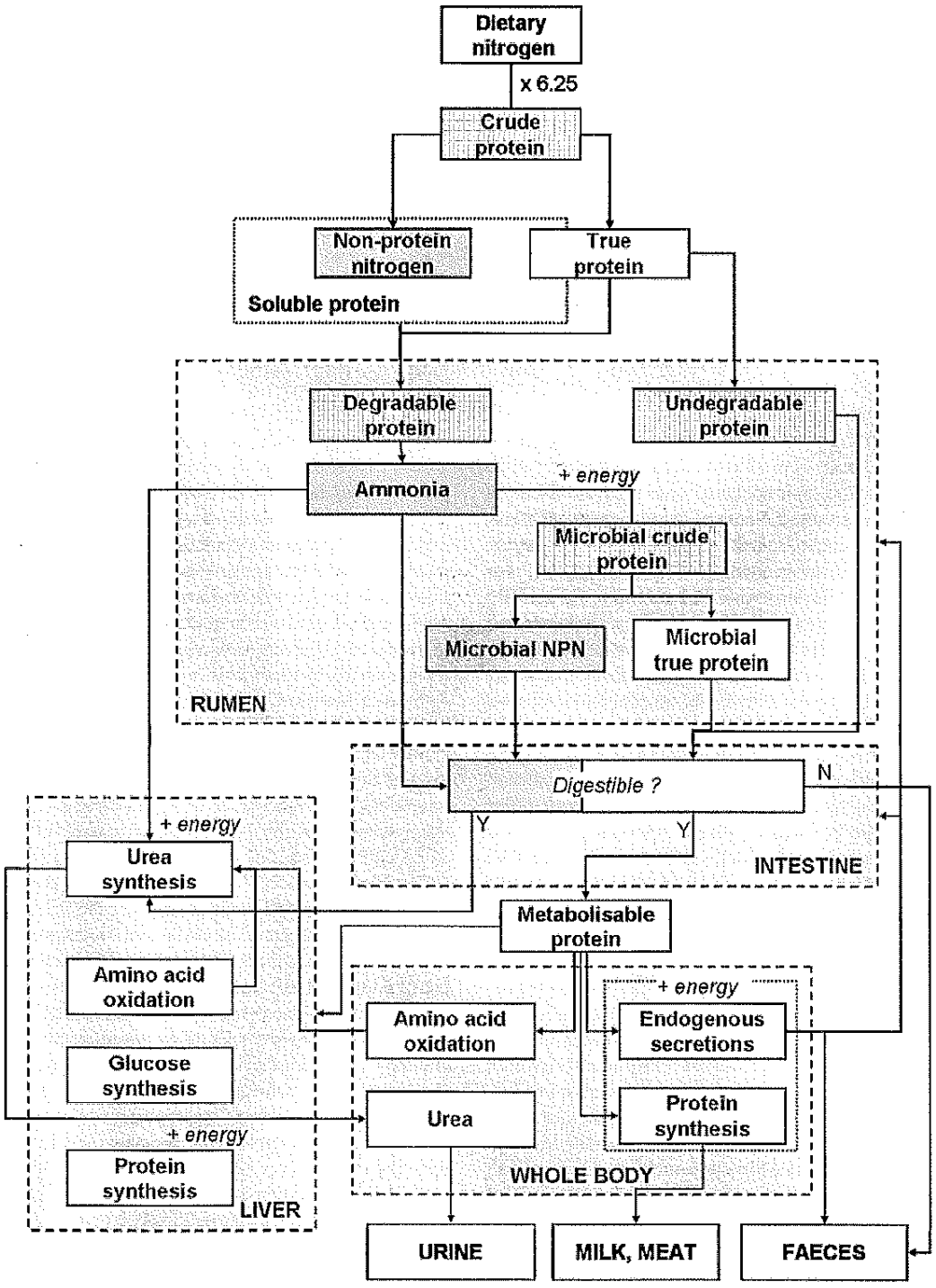


Figure 2.4 Overview of nitrogen metabolism in the ruminant. Sourced with permission: Pacheco and Waghorn (2008).

2.5 Nitrogen use efficiency

The efficiency of N utilisation (nitrogen use efficiency, NUE) is defined as the proportion of dietary N that is used for tissue protein accretion and milk synthesis (Van Vuuren et al., 1993;

Phuong et al., 2013). Generally, the efficiency of N utilisation by ruminants is low, with N efficiencies for lactating dairy cows grazing forage species in the range of 13 - 31 % (protein in product/protein intake) reported (Wanjaiya et al., 1993; Astigarraga et al., 1994; Peyraud et al., 1996; Delagarde et al., 1997; Castillo et al., 2001b). The low NUE occurs because any excess dietary N that is not used for milk production, muscle or conceptus growth cannot be stored and is excreted in faeces and urine. While NUE is ultimately limited by the animals' genetic potential (Rius et al., 2012), three primary causes have been identified for influencing NUE within the animal: (1) total dietary N intake (2) herbage chemical composition and (3) lack of balance or synchronisation of N and energy release in the rumen (West et al., 1997; Castillo et al., 2001b; Phuong et al., 2013).

2.5.1 N intake

The amount of CP in the diet can affect NUE and N excretion (Table 2.1) (Hoekstra et al., 2007a). In intensive Northern Hemisphere systems, cows are fed total mixed rations (TMR; comprising grains, silages and supplements) and the formulation is balanced to provide sufficient N and energy to meet cow requirements. The N included in the diet (often soybean meal or fish meal) is an expensive component, so the amount used is minimised or treated (with heat or chemicals) to limit rumen degradation. Measurement of milk production and milk urea concentration can be used to monitor wastage of dietary N, allowing appropriate modification to the diet to balance N and energy and minimize waste to the environment (Jonker et al., 1998). The ability to match protein supply with animal requirements in New Zealand is more challenging where grazed herbage is the main feed source. Herbage CP content varies depending on climate and management (i.e. harvest interval and N inputs), but is often in excess of dairy cow requirements. Minimum herbage CP concentration required for lactation is ~ 18% (Kolver, 2000; Pacheco and Waghorn, 2008), but swards are generally managed so that their CP concentration exceeds this. When there is an excess of N eaten, NUE decreases as there is little additional milk produced from the additional N intake, leading to greater loss of N in excreta (Tamminga, 1992b; Ordonez et al., 2004). For example, in a grazing study comparing cows consuming herbage containing either 21 or 25% CP, Ordonez et al. (2004) observed no improvement in milk-solids production

when the high N content herbage was fed. Similarly, Beever et al. (1986) demonstrated that while the N intake of cattle grazing white clover (*Trifolium repens* L.) diets was almost twice that of grass diets (due to greater N in the diet and DMI), only 27% more N flowed to the small intestine indicating substantial losses of dietary N before the small intestine. Conversely, NUE is increased by decreasing N intake. However, there will be a point at which decreasing N intake decreases milk production (Calsamiglia et al., 2009), or results in catabolism of tissue protein to meet metabolic demands which is not sustainable (Oldham et al., 1997). Therefore, a balance is sought between providing sufficient CP for production and maintenance but reducing N excretion.

While the amount of N excreted as faeces is relatively constant and increases only slightly with N intake (Jarvis, 1993), the excretion in urine increases exponentially with increasing N intake (Figure 2.5) (Kebreab et al., 2001). Several studies have demonstrated the greater partitioning of dietary N to urine compared with faeces and milk (Table 2.1). Thus the potential for reducing N loss and increasing NUE lies largely with reducing N excreted in urine.

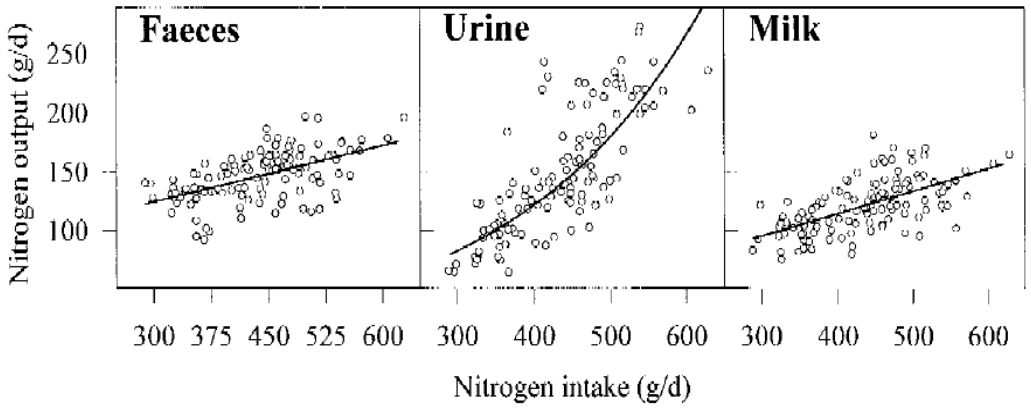


Figure 2.5. Relationship between total N intake and faecal, urinary and milk N outputs. Sourced with permission: Kebreab et al., (2001).

Table 2.1 Nitrogen intake, urine N concentration and N partitioning of dairy cows fed diets of forage species.

				N partitioning (g/cow/d)			
Reference	Species	N intake (g)	Urine N (g N/L)	Milk N	Faecal N	Urine N	**% N to urine**
Wanjaiya *et al*. (1993)	*Trifolium repens*	480		64	165	251	**52**
Van Vuuren *et al*. (1993)	*Lolium perenne*	500		140	195	170	**34**
	L. perenne[1]	423		109	110	204	**48**
Astigarraga *et al*. (1994)	*L. perenne*[2]	503		109	113	281	**56**
Peyraud *et al*. (1996)	*L. perenne*[1]	489		112	116	261	**53**
	L. perenne[2]	315		98	102	115	**37**
Delagarde *et al*. (1997)	*L. perenne*	584		119	118	348	**60**
	L. perenne/T. repens	466		68	151	200	**43**
Woodward *et al*. (2012)	Multi species sward[3]	350		79	136	100	**29**
	L. perenne/T. repens	590	5.7	105		438	**74**
Totty *et al*. (2013)	Multi species sward[3]	551	3.4	112		354	**64**
	L. perenne/T. repens	366		54	132	167	**46**
	L. perenne/T. repens + Medicago sativa	408		53	113	229	**56**
Waghorn *et al*. (submitted)	*L. perenne/T. repens + M. sativa + Plantago lanceolata*	440		68	138	220	**50**
	L. perenne/T. repens	604	5.4				
	50% L. perenne/T. repens + 50% P. lanceolata	584	3.6				
Box *et al*. (2016)	*P. lanceolata*	593	2.4				
	L. perenne	560	7.0				
	80% L. perenne/T. repens + 20% Cichorium intybus	512	5.7				
	60% L. perenne/T. repens + 40% C. intybus	464	4.3				
	80% L. perenne/T. repens + 20% P. lanceolata	496	6.7				
Minnee *et al*. (2017)	*60% L. perenne/T. repens + 40% P. lanceolata*	432	5.0				

[1] Low rate of N fertiliser applied to sward.
[2] High rate of N fertiliser applied to sward.
[3] Multi species swards including forb and legume species.

2.5.2 Feed chemical composition

2.5.2.1 Nitrogen

The N content of feeds is commonly reported as CP, which equals N × 6.25 (as protein is on average 16% N). This measure assumes that the N in herbage is largely as proteins, and discounts NPN forms. The true nutritional value of the herbage N is influenced by the distribution of N amongst components (i.e. "fractions"). As discussed in section 2.4, N in herbage is categorised into NPN and TP. Non-protein N usually account for 20 – 30% of herbage N (Mangan, 1982; Bryant et al., 2012), and are largely contained within the cell contents and so are rapidly released and degraded when cell rupture occurs (Ferguson and Terry, 1954). Whereas, TP are molecules comprised of about 20 different amino acids linked by peptide bonds. True protein accounts for 75 – 90% plant N (Waghorn and Clark, 2004). True protein can be further defined according to its solubility in the rumen. Fraction 1 proteins are those that are water soluble, and so are also referred to as "soluble proteins". Ribulose-1, 5- biphosphate (Rubisco) makes up the majority (~ 70%) of soluble protein and is contained in chloroplasts but readily released once cell rupture occurs. Fraction 2 are termed "insoluble degradable protein" which are less accessible to rumen microorganisms and are thus more slowly degraded (3 – 30% per hour). Fraction 2 proteins are mixed proteins that account for approximately 25% of TP, and occur in the chloroplasts and cytoplasm of the cell (Mangan, 1982). A further fraction of N is defined as "Undegradable" or "C fraction" that is not degraded in the rumen. The C fraction is largely associated with cell walls, and account for 5 – 15% of CP ingested. Thus, the proportion of the N fractions in the feed and the rate in which they are degraded will influence the rate at which ammonia accumulates in the rumen (Figure 2.6) which may then in turn influence N excretion (section 2.4). For example, Kebreab et al. (Kebreab et al., 2001) demonstrated a 24% decline in urinary N excretion when cows were fed diets of low protein degrability compared with high protein degrability, with no adverse effect on milk production.

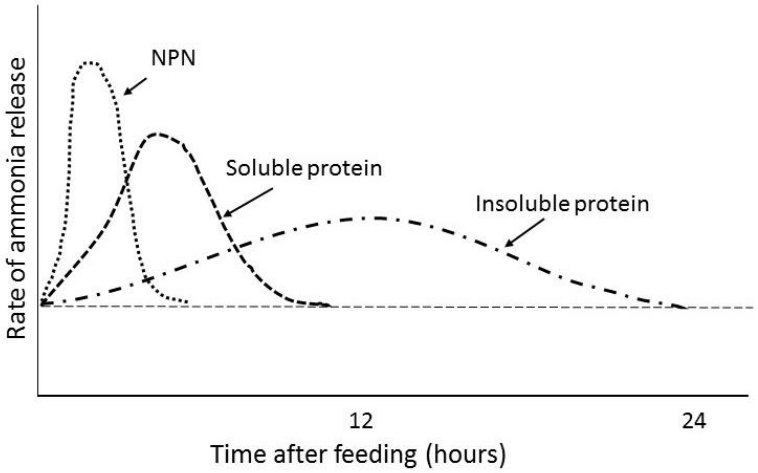


Figure 2.6 Theoretical intra-ruminal rates of ammonia release from non-protein nitrogen (NPN), soluble and insoluble proteins after eating forages. Adapted from: Johnson (1976).

2.5.2.2 Carbohydrates

Carbohydrates (CHO) form the majority of DM in plants, and the efficiency of MPS is dependent on the supply and rate of CHO fermentation (Smith, 1975). As discussed further in section 2.6.1, the chemical composition of CHO differs considerably between forage species and tissue types, which affects their degrability. Carbohydrates can be grouped into two categories: (1) structural carbohydrates (SC) that form cell walls and (2) non-structural carbohydrates (NSC). The NSC are simple sugars, starch and fructans and are major sources of energy for high producing dairy cattle (NRC, 2001). Non-structural carbohydrates are rapidly (> 400 %/h) and almost completely fermented in the rumen (Weisbjerg et al., 1998), so provide a ready source of energy for MPS. When supply of NSC is limiting, microbial growth and MPS is reduced as microbes must obtain their energy from the fermentation of SC, thus concomitantly NUE may also be reduced (Van Soest, 1994) (discussed further in section 2.5.3 and 2.6.1).

2.5.3 Balancing N and energy supply

Balancing nutrient supply (synchrony) involves considerations of the total supply of nutrients, and the rate at which these nutrients become available in the rumen (i.e. by cell rupture and solubilisation or degradation). When there is ready supply of energy (mainly NSC, released by cell rupture) dietary N can be incorporated into microbial N by MPS and used for production (section 2.4). Conversely, a lack of readily available energy, alternative energy sources must be

obtained by the microbes through the slower degradation of SC, potentially reducing the rate of MPS and leading to an accumulation of ammonia in the rumen (Kebreab et al., 2001; Hoekstra et al., 2007a). In grazed herbage, generally, the amount of soluble protein is high and NSC content is low and the imbalance results in poor NUE (Rearte, 2005; Belanche et al., 2013). Or as Beever and Cottrill (1994) suggested, the low NUE of cows fed herbage may be due to the N and the energy from herbage becoming available in the rumen at different times during digestion. Thus, it can be postulated that the rate at which plant cells are ruptured and degraded during the ingestion and ruminal digestion phase could alter the supply of N and energy to the rumen, and then the efficiency of utilisation of N and energy.

While studies have shown that synchronous diets can improve MPS or reduce rumen ammonia concentration *in vivo* and *in vitro*, often these have failed to improve animal productive performance (Herrera-Saldana et al., 1990; Sinclair et al., 1993; Kolver et al., 1998). However, few studies have investigated synchrony with the goal of reducing N excreted in the urine. Understanding the pattern of CP and CHO release and degradation from herbage may help explain differences in urinary N excretion observed in studies that cannot be explained by N intake alone (Woodward et al., 2012; Minneé et al., 2017). Further, in a review by Edwards et al. (2007) investigating the influence of breeding perennial ryegrass for high water soluble carbohydrate (WSC) concentration, there was little consistent effect on milk yield, but lower urinary N excretion that was found to be closely correlated with the ratio of CP and WSC in herbage.

2.6 Factors that influence herbage digestion

As described in section 2.3, digestion of herbage involves firstly ingestion, and then ruminal and intestinal degradation. Several studies have investigated the variation in rate and extent of ruminal degradation between feeds to determine the main feed characteristics that influcence physical and microbial digestion of herbages, but less is known about the effects of ingestion on herbage degradation.

A study by Beever et al. (1986) explored digestion of perennial ryegrass and white clover and showed significant forage species effects on nutrient supply to the rumen due to variation in the

physical characteristics and chemical composition of the herbage, because both influence how the herbage is ingested and degraded. Expanding upon this, Burke (2004) defined the ruminal degradation and fermentation kinetics of 23 fresh and conserved feeds after mechanical maceration. The amount of material (dry matter) initially solubilised ("A" fraction) ranged from 21 – 55%, and subsequent rate of degradation (k) of herbage DM ranged from 5 – 26%/h between species. For example, Figure 2.7 illustrates the variation between species in degradation (loss) of fibre (as neutral detergent fibre, NDF) over time. Research has sought to determine what factors influence degradation, and concluded that it is a complex process that this influenced by both forage species and the animal. It could be proposed that the same factors that influence ruminal degradation also influence how herbage is degraded during ingestion.

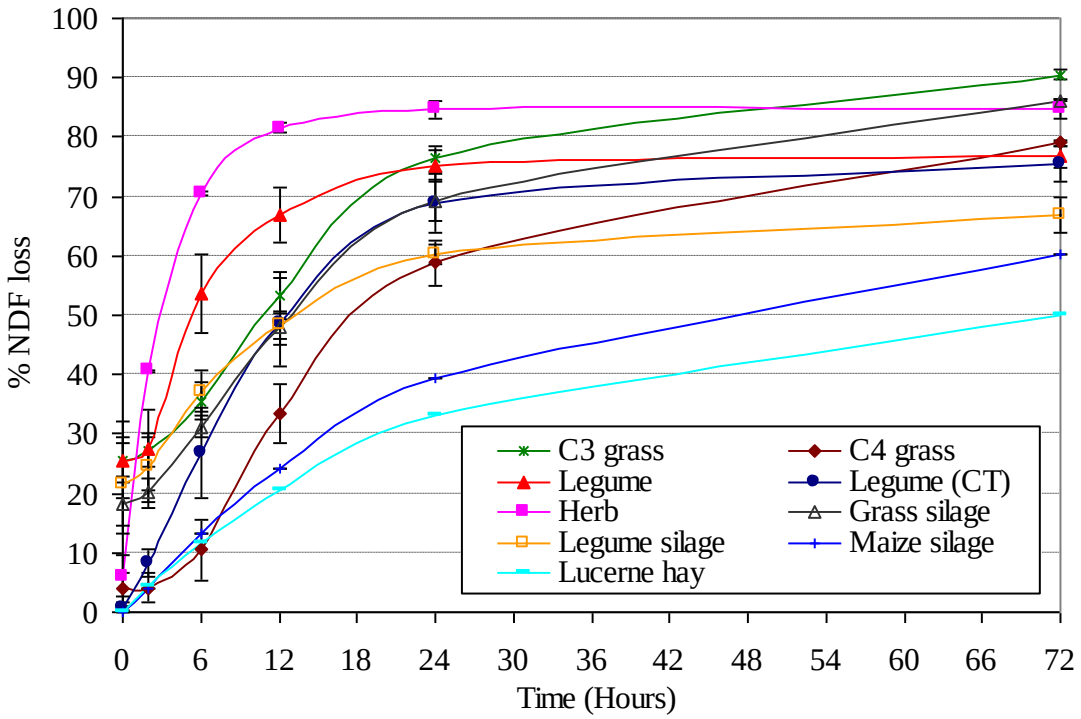


Figure 2.7 Neutral detergent fibre (NDF) degradation curves for eight forage types evaluated *in sacco*. Bars are standard errors of the mean at each time; data sourced with permission from Burke (2004).

2.6.1 Chemical composition of the cell walls

The cell walls of forage species are complex three-dimensional structures comprised of polysaccharides (cellulose, hemicellulose and pectin), protein and lignin (Aman, 1993). The chemical composition of cell wall varies between species, and within a plant (i.e. the different

tissue types). Cellulose is a straight chain polymer of glucose units that is more resistant to bacterial degradation than hemicellulose, a branched polymer of several different sugars that can be extensively degraded by most ruminal bacteria depending on the extent of binding with lignin (Schwartz and Gilchrist, 1974). Thus the composition and configuration of cell walls affects the rate and extent of degradation (Evans, 1967b; Tamminga, 1986; Buxton and Fales, 1994). While Burke's (2004) study reported that herbage containing greater fibre (cell wall, NDF) concentrations degraded at slower rates than herbage with lesser fibre concentration, the study showed that the relationship between NDF concentration and degradation rate was not strong (R^2 = 0.50). This indicates while concentration of cell wall fibre is important, it is not the only factor influencing degradation. And thus, the concentration of fibre cannot solely infer how degradable a feed is. For example, the digestible fibre of legumes is rapidly digested, but legumes contain more indigestible fibre than grass. Meaning that overall, ruminants typically digest 60 – 70% of grass fibre but only 40 – 50% of legume fibre (Varga and Prigge, 1982; Buxton and Redfearn, 1997). Differences in the degradation rate of fibre between C_3 and C_4 grasses is also attributed to variation in cell wall composition. Compared with C_3 species, C_4 grasses have greater amounts of thick-walled sclerenchyma and vascular tissue, and these structures impede degradation (Wilson et al., 1989a; Wilson, 1993). This was illustrated by Burke (2004) who reported NDF degradation rates (k) of 9.3%/h for perennial ryegrass (C_3) compared with 5.8%/h for kikuyu (*Pennisetum clandestinum*) a C_4 grass.

The digestibility of the cell walls of grasses is negatively correlated with the concentration of lignin (Wilman and Altimimi, 1984). Waghorn and McNabb (2003) described lignin as the "glue" holding structural fibres together. Lignin is largely indigestible and water-resistant, and as such it acts as a physical barrier to microbial degradation. However, the digestibility of lignin and degree of lignification also differs between species. In grasses, many of the cell types may become lignified, whereas in legumes only the cells in the vascular tissue become lignified. However, the lignified secondary cell walls of grasses are digestible, but those of legumes are largely indigestible (Wilson, 1993), so inferring degrability from lignin concentration is complex.

2.6.2 Herbage biomechanical properties

The biomechanical properties of herbage are relevant to studies of herbage nutritive value, as these can influence resistance to degradation and animal preference (Sanson et al., 2001). The strength and toughness of ryegrass herbage for example has been associated with preference, intake rate, and production (MacKinnon et al., 1988; Inoué et al., 1994; Bryant et al., 2008). A reduction in acceptability is likely associated with the increased difficulty of grazing tougher herbages due to increased resistance to prehension and comminution (Ungar and Noy-Meir, 1988; Laca et al., 1992) as well as reduced rates of ruminal degradation (Wilson et al., 1989a). In an effort to measure herbage toughness, laboratory assessments of the energy required to fragment plant material have revealed considerable differences between species, i.e. grasses are tougher and require more energy to fragment than legumes; within grasses, C_4 species are tougher than C_3 species (Wilson et al., 1989a), and within species differences between cultivars and stages of maturity of ryegrass have been reported (Evans, 1967b; Bryant et al., 2008; Sun et al., 2010). Wilson et al. (1989a) and Akin (1986) suggest differences in carbohydrate composition and anatomy (section 2.6.1) effect the resistance to degradation (resistance to shearing) between species. What is not known, is how the biomechanical properties and extent of comminution affects nutrient release from herbage. Furthermore, there is a lack of knowledge regarding the biomechanical properties of other common forage species such as legumes and herbs, and how these might influence their degradation during ingestion and ruminal degradation.

It could be expected that herbage that is physically strong would result in less immediately solubilised material (A fraction) following mastication or maceration than weaker herbage. Yet this is not necessarily the case, studies by Burke (2004; Table 2.2 and Figure 2.8) and Minnée et al. (2017) showed that CP solubilised in the A fraction was up to 30% greater from ryegrass than from chicory (*Cichorium intybus*) herbage which is generally regarded to have less fibre than ryegrass. This may be because the ryegrass herbage required more force to macerate than chicory herbage resulting in a greater proportion of cells damaged, but this theory has not been tested. Lee and Pearce (1984) showed that conserved forages that had the greatest reduction in particle size during mastication by steers, also contained the greatest concentration of fibre and required

the greatest amount of energy to macerate. Furthermore, in studies with livestock fed fresh herbage, grasses containing greater content of structural tissue required more mastication (Beauchemin, 1991) and were fragmented to a greater extent than grasses with lower NDF concentration (Poppi et al., 1981b; Wilson et al., 1989a), which theoretically, could release more nutrients (CP and CHO) from herbage. Further investigation is required to determine the extent of biomechanical differences between forage species, and to what extent this effects nutrient release during comminution. This will aid our understanding of nutrient utilisation in the rumen from contrasting forage species. Further research is required to understand the fragmentation of forages during masticating and what plant characteristics influence this, and how this influences nutrient availability and subsequent digestion.

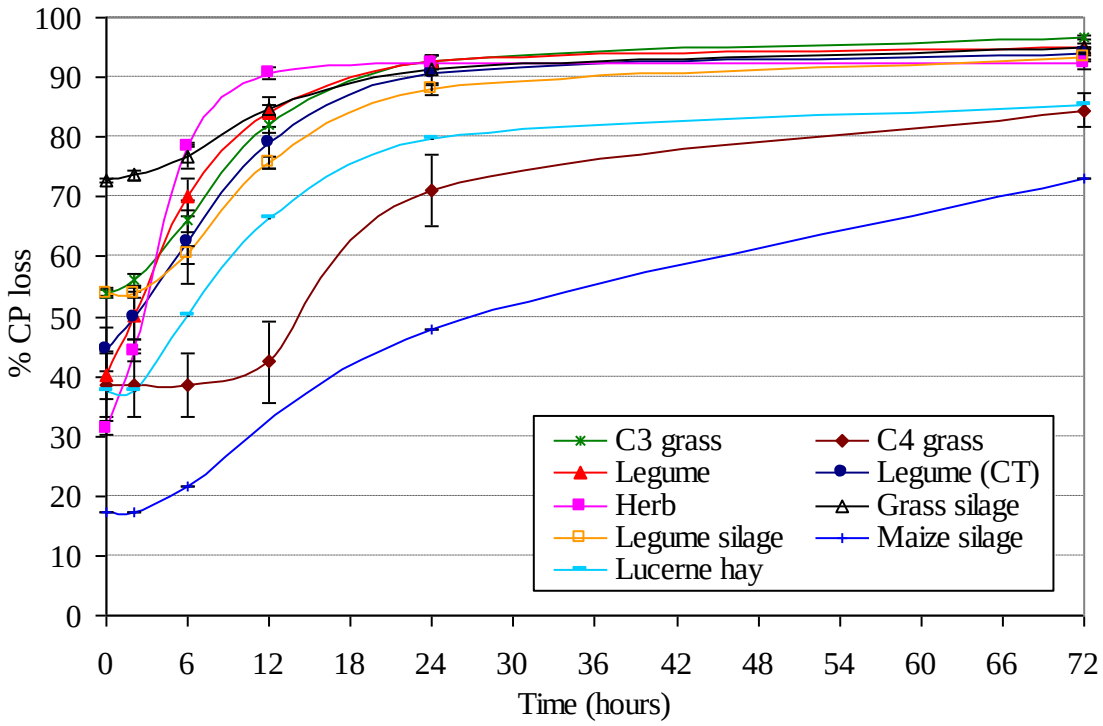


Figure 2.8 Crude protein (CP) degradation curves for eight forage types evaluated *in sacco*. Bars are standard errors of the mean at each time; data sourced with permission from Burke (2004).

Table 2.2 Crude protein (CP) concentration in the DM (g/100 g DM), and distribution between the soluble (A) and degradable insoluble (B) fractions, as well as fractional degradation rate (k, %/h) and lag time (l; hours) of *in sacco* incubations with fresh (frozen) forages minced to achieve a particle size to resemble rumen contents. From Burke, with permission, (2004).

Species	CP	A	B	k	l
Perennial ryegrass	15.5	52.2 ± 0.29	43.5 ± 0.46	15.0 ± 0.60	4.6 ± 0.12
Cocksfoot	23.7	55.0 ± 2.42	41.1 ± 3.80	15.9 ± 5.2	5.0 ± 0.86
Tall fescue	16.4	53.0 ± 1.94	43.1 ± 3.18	7.5 ± 1.7	3.2 ± 1.35
Yorkshire fog	23.7	54.8 ± 1.63	43.6 ± 2.28	8.7 ± 1.1	1.0 ± 0.66
Prairie grass	19.9	52.4 ± 0.48	43.0 ± 0.61	17.1 ± 0.70	0.9 ± 0.12
Grasslands Tama	21.3	56.4 ± 1.22	41.7 ± 1.97	13.9 ± 2.4	4.3 ± 0.58
Kikuyu	16.4	47.9 ± 0.74	41.5 ± 1.12	12.5 ± 0.60	10.7 ± 0.42
Paspalum	13.5	29.1 ± 2.44	20.9 ± 6.54	7.8 ± 1.2	11.6 ± 1.93
White clover	26.9	38.4 ± 1.45	58.0 ± 1.81	19.1 ± 1.64	1.3 ± 0.22
Lucerne	29.9	52.0 ±1.48	41.5 ± 1.81	15.3 ± 1.7	0
Red Clover	27.4	30.1 ± 5.42	65.4 ± 7.33	10.4 ± 3.2	0
Lotus corniculatus	22.2	51.1 ± 1.46	44.3 ± 1.88	15.0 ± 1.7	1.0 ± 0.37
Lotus pedunculatus	21.5	33.0 ± 1.35	58.0 ± 2.24	11.4 ± 1.4	4.8 ± 0.45
Sulla	23.0	49.5 ± 2.34	46.4 ± 3.11	11.7 ± 2.3	0
Chicory	19.3	29.3 ± 1.14	65.4 ± 1.38	27.3 ± 2.1	0.8 ± 0.15
Plantain	24.7	33.3 ± 7.80	57.1 ± 8.57	34.9 ± 13.1	1.6 ± 0.60

2.6.3 Herbage stem : leaf ratio

At the interface between plant and animal, there is more than merely chemistry influencing release of nutrients. Physically, different forage species present themselves in different ways within the sward, and management can have a profound effect on physical characteristics of swards. Forage management for grazing usually aims to maximise the proportion of grazeable green leaf and minimise stem material in the sward, as the latter is of lesser nutritional value. The primary function of laminae (leaf) is to generate energy for the plant via photosynthesis, and thus most plant N is found in the leaf (Wilman and Altimimi, 1982; Grindlay, 1997). Green leaf tends to be thinner and weaker than other plant parts (Waghorn and Shelton, 1988) as the dominant tissue types are the thin-walled, loosely arranged mesophyll cells (e.g. parenchyma cells in the ground tissue) (Buxton and Redfearn, 1997). Conversely, plant sheath and stem provide mechanical support for the leaf,

and transport material (i.e. nutrients and water) around the plant, hence these structures have a greater proportion of thick walled and lignified cells and conductive tissue than leaf, and are more resistant to ruminal degradation (Buxton and Redfearn, 1997; Chaves et al., 2006). Therefore, the relative proportion of stem and leaf will influence the potential degrability of the herbage. Investigating the degradation rate of five temperate grass species, Chaves et al. (2006) demonstrated that stem was degraded at a quarter of the rate of leaf (k = 0.025 vs 0.105 %/h). The authors suggested the reduced degradation rate was in part due to variation in the extent of particle size reduction and release of cell contents of the mechanically macerated material used in the study. Knowledge of whether this effect applies to dicotyledonous and herbaceous species is required to better understand their degradation and utilisation by ruminants.

2.6.4 The animal

The physical presentation and characteristics of herbage influences feed intake rate, total intake and the effort required by the animal to ingest and degrade the herbage (John and Reid, 1986; Wright and Illius, 1995; Pérez-Barbería and Gordon, 1998). For example, Gregorini et al. (2013b) observed the grazing behaviour of dairy cows on perennial ryegrass or chicory based pastures and showed those grazing chicory spent more time masticating the herbage than cows grazing ryegrass. The opposite was observed by Ulyatt et al. (1986) who showed sheep chewed more during eating perennial ryegrass compared to legumes, e.g. performing 37 vs 13 chews/g DM from perennial ryegrass and red clover respectively. The findings of Gregorini et al (2013) may have indicated a greater extent of oral manipulation of chicory herbage to form a bolus for swallowing rather than the fragmentation of the herbage into small particles, but this was not measured.

The fragment size, or particle size distribution (PSD) of masticated herbage is influenced by four factors: teeth (chewing effectiveness, i.e. how effective they are at cutting, crushing or grinding the plant material), ingestive behaviour (bite rate, time spent chewing etc.), volume of feed ingested, and the physical characteristics of the herbage (Pérez-Barbería and Gordon, 1998). There are large variations in feed fragment size during ingestion between animals (Lee and Pearce, 1984; Pérez-Barbería and Gordon, 1998; Acosta et al., 2007) so it is challenging to isolate the characteristics of

the herbage that influence this. However, animals are able to compensate for lack of chewing effectiveness by increasing the number of jaw movements per amount of herbage processed to achieve an appropriate PSD in swallowed boli. Alternatively, the number of jaw movements could be maintained, with larger fragments of swallowed herbage which would then require greater ruminal processing and/or retention time (Pérez-Barbería and Gordon, 1998). These two processes would have very different patterns of herbage nutrient release. For example, in a study comparing the degradation of C_3 and C_4 grasses, it was found that despite the greater toughness of *Panicum* sp., it was fragmented to a greater extent than ryegrass during mastication (Wilson et al., 1989b). The more extensive comminution was likely necessary to form a bolus enabling the tougher C_4 herbage to be swallowed.

Poppi et al. (1981b) found that fragment size reduction was greater with increased maturity in grasses, which tend to have greater SC and are tougher than immature herbage. However, Boudon and Peyraud (2001) found that despite differences in eating time between ryegrass diets of different maturities, nutrient release was similar. They did not report fragment size so no relationship between degree of comminution and nutrient release could be ascertained. Waghorn et al. (1989) showed that the extensive reduction in fragment size when lucerne was fed to cows, coupled with the high content of soluble DM of the feed led to a rapid increase in rumen VFA and ammonia concentrations and a decline in rumen pH. Proportionally larger numbers of microbes adhere to small than to medium particles, which could increase digestion rate (Worrell et al., 1986). On the other hand, extensive reduction of fragments to very small particle size could result in a faster rumen clearance, enabling more undegraded fibre and protein to pass out of the rumen (Kennedy and Murphy, 1988; Buxton and Redfearn, 1997; Noziere et al., 2010). Thus, it is plausible to hypothesize that the extent of fragmentation would influence digestion. How the extent to which fragmentation influences nutrient release and subsequent digestion, remains to be defined.

2.7 The potential to manipulate herbage degradation through nitrogen fertiliser management

The application of nitrogen fertiliser results in increased herbage production in most non-leguminous forages (Waite, 1965; Reid, 1986). Several studies have described effects of fertiliser (inorganic) N application on herbage physical characteristics and chemical composition. Nitrogen fertilisation increases N concentration in grass species (Ferguson and Terry, 1956; Goswami and Willcox, 1969; Hoekstra et al., 2007b) through plant uptake and storage, but also by increasing the leaf: stem ratio of the plant as leaf material has greater N content than stem material (Waite, 1965). At high N application rates (> 300 kg N/ha/y) increasing total N content in grass is accompanied by a rise in the proportion of soluble NPN compounds in herbage (particularly nitrate N) (Ferguson and Terry, 1956; Reid and Strachan, 1974; Waghorn and Clark, 2004) and likely result in increased rumen ammonia concentrations and reduced NUE (section 2.4 and 2.5). Furthermore, in grasses, increasing fertiliser N application reduces NSC concentration (Reid and Strachan, 1974; Hoekstra et al., 2007b), because increased herbage growth utilises more NSC for photosynthesis and converting plant nitrate into proteins (Reid and Strachan, 1974) which compound reductions in NUE. Conversely, Hoekstra et al. (2007b) showed that fibre concentrations were greater in grasses receiving low N application rates (0-90 kg N/ha/y) which reduce herbage digestibility (section 2.5.2). All these factors are able to influence the extent of herbage comminution and nutrient release during ingestion, but the impact of N fertiliser application rates on these factors has not been measured specifically. Furthermore, there is a paucity of information regarding the effect of increasing N fertiliser application rates on the physical and chemical characteristics of herbage species, and an improved understanding may be used to inform fertiliser management for improved NUE.

2.8 Conclusion

The digestion of forage species by ruminants has been described and the factors that influence herbage ingestion and ruminal degradation discussed. A considerable portion of the known literature has focussed on the digestion of forage grass species, and there is a need to extend this

knowledge to encompass other common forage species. Furthermore, while much is known about the ruminal degradation of herbage, less is known about the degradation of herbage and release of nutrients during ingestion. Improving knowledge on how forages are degraded during ingestion and the particular characteristics of the herbage that influence this would aid our understanding of why differences in NUE occur, and indicate means to improve NUE and decrease N excreted in urine.

3

Source of herbage material and techniques for assessing the physical strength of herbage

3.1 Introduction

Dairy farming in New Zealand is based on the rotational grazing of swards comprised of perennial ryegrass sown with white clover. These swards are relatively high yielding and generally meet the nutritional requirements of dairy cattle. However, they often contain crude protein concentrations in excess of animal requirements, which contributes to high urinary nitrogen (N) excretion and thus to nitrate leaching (Chapter 2). Consequently, research effort is being directed toward identifying alternative forage species and management practices that can reduce nitrate leaching while maintaining herbage dry matter yield (DMY) and animal production (Gregorini et al., 2016). Several forages have been identified as options because, compared with ryegrass/white clover, they have a lower requirement for fertiliser N to meet similar yields (Martin et al., 2017), greater rates of uptake of N from soils (Woods, 2017), reduced forage N content and greater WSC content (Minneé et al., 2017), or are utilised more efficiently by livestock.

The research reported in this thesis examined the chemical and physical characteristics of up to five forage species (chicory, lucerne, plantain, ryegrass and white clover) and how the characteristics of herbage from these forages influence degradation and nutrient release during ingestion. A review of the literature (Chapter 2) identified that chemical and physical characteristics of a forage species can affect the intake, comminution and degradation of that species when eaten, and revealed that published quantitative data on these characteristics are limited. While the chemical composition of herbage is commonly used for inferring forage species nutritive value (NV), and laboratory techniques for measuring chemical composition are well established, the physical (biomechanical) properties of herbage are seldom considered and methods for measuring them are poorly developed.

The objectives of this chapter are to:

- Describe in detail how the forages used in this study were established and managed.
- Describe the criteria and methods of harvesting and sample collection.
- Report investigations into techniques that can detect differences in the biomechanical properties of forages. The techniques selected focussed on two properties:
    - The force required to punch or fracture herbage material which is a measure of material strength.
    - The energy required to macerate herbage material as a measure of herbage 'toughness'.

3.2 Source of herbage

3.2.1 Experimental design

Herbage material used in the studies described in this thesis were collected from the 'Critical N' field plot experiment which was part of a larger programme of research (Forages for Reduced Nitrate Leaching, FRNL). The experiment was established on a non-irrigated site at Newstead, near Hamilton, New Zealand (37°76'S, 175°36'E; 40 m.a.s.l). The 'Critical N' experiment comprised of seven swards (five monocultures and two mixed pastures) and six rates of fertiliser N application (0, 50, 100, 200, 350 and 500 kg N/ha/y; hereafter termed 0N, 50N … 500N). Sward treatments were: monocultures of perennial ryegrass (*Lolium perenne* L., cv. One50 with AR37 endophyte), white clover (*Trifolium repens* L., cv. Mainstay), lucerne (*Medicago sativa* L., cv. Torlesse), chicory (*Cichorium intybus* L., cv. Choice), and plantain (*Plantago lanceolata* L., cv. Tonic) monocultures; a 'Standard' mixture of perennial ryegrass with white clover; and a 'Mixed pasture' sward including all forage species (forage). Only the monoculture swards and four of the six N application treatments were used in the research presented here. Details of cultivars, sowing rates and fertiliser N application rates are presented in Table 3.1.

Table 3.1 Forage, cultivar, sowing rate, number of harvests/annum and N application after each harvest for the 0, 100, 200 and 350 kg N/ha treatments that were used in research presented here.

					N application rate (kg N/ha) after each harvest			
Forage	Cultivar	Source	Sowing rate (kg seed/ha)	No. of harvests/y	0	100	200	350
Perennial ryegrass	One50 (AR37 endophyte)	Agricom	20	10	0	10	20	35
White clover	Mainstay	Agricom	8	6	0	16.7	33.3	58.3
Lucerne	Torlesse	Agricom	14	8	0	12.5	25	44
Chicory	Choice	Agricom	8	8	0	12.5	25	44
Plantain	Tonic	Agricom	10	10	0	10	20	35

Treatments were arranged in a randomised split plot design with forage as main plots and N fertiliser application rates as sub-plots (Figure 3.1), in three blocks (replicates). An example of one block is given in Plate 3.1.

Nitrogen fertiliser sub-plots (9 m long x 6 m wide) are randomised along the length of each main plot. Plots were not irrigated, and were harvested under a cut and carry system. Soil type at the site is a Horotiu silt loam (Singleton, 1991), a Typic Orthic Allophanic soil under the New Zealand classification system (Hewitt, 1998) or Typic Udivitrand under the USDA classification system. Analysis of soil samples collected to a depth of 150 mm from the site in September 2014 revealed a pH of 5.8 (1:2.1 v/v soil-water slurry) and mineral concentrations of 32 µg/ml P (Olsen), 5 K and 13 Mg (MAF QT units) and 62 ppm sulphate-S (phosphate extraction).

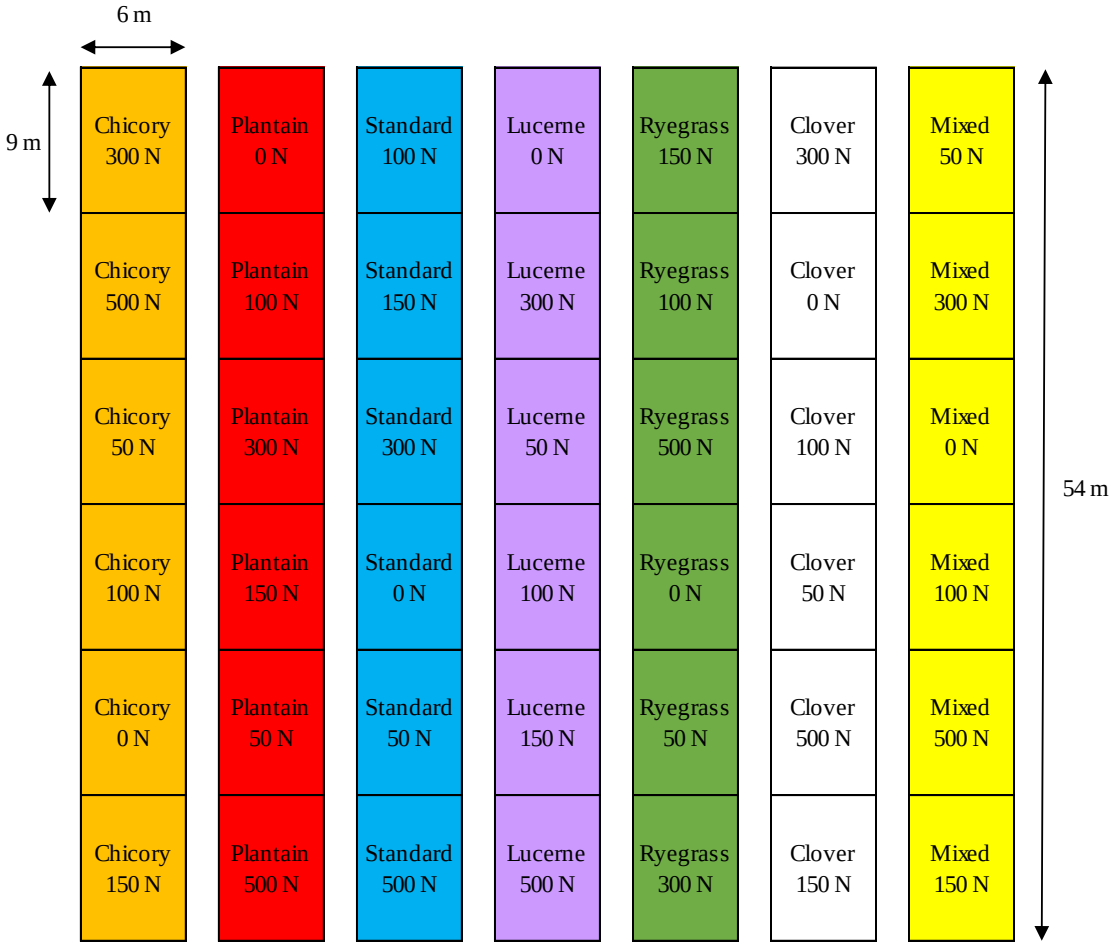


Figure 3.1 Example of the layout of one block of the Critical N study. Main plots (sward type) are denoted by different colours.

Plate 3.1 A view of one replicate of the experiment showing sward main plots (54 m long and 6 m wide).

3.2.2 Climatic conditions

Total monthly and annual rainfall, and mean minimum and maximum air temperature (°C), over the duration of the experiments (2015 calendar year) are illustrated in Figure 3.2 along with the long-term 30-y mean. Total annual rainfall during the experiment was less than the long-term mean, largely due to reduced rainfall in summer and winter. Rainfall was variable in autumn and spring, with well above average rainfall in April, September, and November but very low rainfall in October (60% less than the 30-y mean). Air temperatures were generally warmer than the long-term mean, particularly in January where the mean average temperature was 20.1 °C compared to the 30-y mean of 18.6 °C.

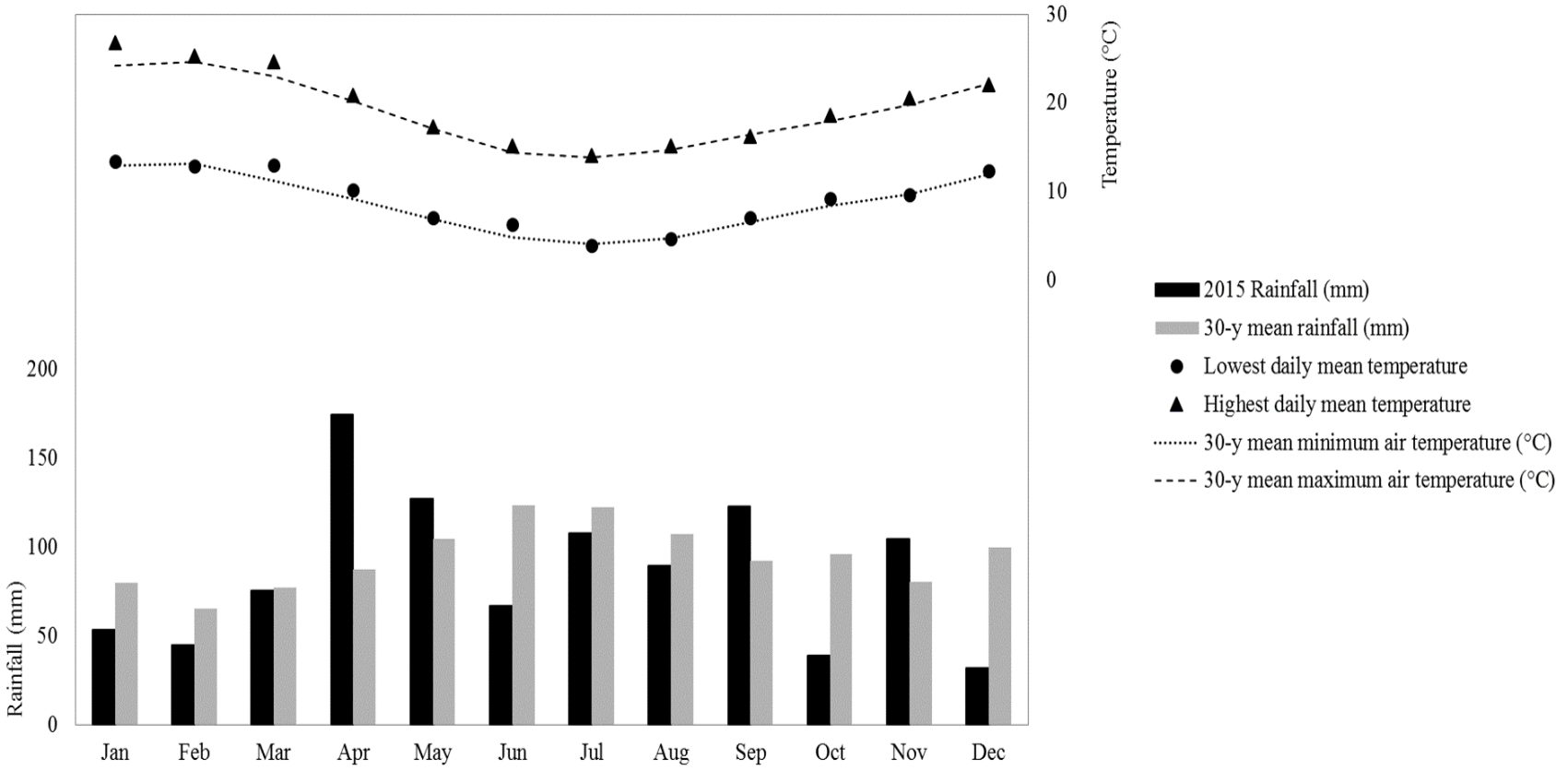


Figure 3.2 Total monthly and annual rainfall (mm) and mean maximum and minimum air temperatures (°C) each month during the experimental period (2015) and the long term 30-y average. Data recorded at the Ruakura Meteorological station, 5 km from trial site.

3.2.3 Management of swards

3.2.3.1 Establishment and maintenance of swards

Existing perennial ryegrass-white clover swards were removed using two applications of 4.25 L/ha Glyphosate 510SL (glyphosate 510 g a.i/L as the isoproplyamine salt; Agpro, Auckland) on 4 September and on 1 October 2014. On 13 October, the site was ploughed with a mouldboard plough to a depth of 15 cm and 1.5 t/ha of granulated lime with a fertiliser mix (700 kg/ha Serpentine super 15K™ and 150 kg/ha Diammonium phosphate, DAP; 18N: 68P: 15K: 60S: 35Mg: 116Ca; Ballance Agri-nutrients, Tauranga) was incorporated into the soil using a power harrow to amend soil fertility on the basis of the soil test results and recommended fertiliser application. The site was then rolled with a Cambridge roller, and on 24 October, main plots were sown with the relevant seed mixtures to a depth of 5 mm using a roller drill with 100 mm row spacing. Endure slug-bait (7.5 kg/ha) was applied as precaution to protect seedlings from slug damage.

A post-emergence herbicide, Glyphosate 510SL (4.25 L/ha, glyphosate 510g a.i/L), was applied the day after sowing to eradicate weed seedlings that had germinated between cultivation and sowing.

Broadleaf weeds were controlled in chicory, plantain and perennial ryegrass swards by applying 1.0 L/ha Headstart™ (flumetsulam 50 g a.i/L in an oil dispersion; Zelam, New Plymouth) on 3 December. At the same time, Sequence™ herbicide (0.25 L/ha, clethodim 240 g a.i/L; Syngenta, Basel, Switzerland) was applied to control grass weeds in chicory, lucerne, plantain and white clover swards. A herbicide mixture of 1.0 L/ha of Sequence™ with 3 L/ha Dynamo™ (flumetsulam 17 g a.i/L with bentazone 480 g a.i/L; Zelam, New Plymouth) was applied to white clover swards in January 2015, lucerne swards in June 2015 and to both white clover and lucerne in October 2015.

3.2.3.2 Fertiliser

Gromore brassica™ (Grochem, Porirua) was applied in December 2014 to supply trace minerals in a liquid foliar fertiliser at 10 L/ha (contains: 390g/L elemental Nitrogen as urea 204g/L, ammonium 93g/L and nitrate 93 g/L, with 2.5 g/L Boron and 0.05 g/L Molybdenum). In April 2015 a maintenance fertiliser mixture of 45.5% Superten (9P: 10.5S: 22Ca), 45.2% Serpentine super (6.8P:

8.4S: 5Mg: 16.5Ca), 6.4% AgSalt Grade 10 and 2.7% Sulphurgain™ was applied at a rate of 550 kg/ha with 400 kg/ha of granulated lime. In September 2015, maintenance K fertiliser was applied at 150 kg K/ha with 500 kg/ha of granulated lime. Nitrogen fertiliser treatments were applied by hand as SustaiN™ (N46; Ballance Agri-nutrients, Tauranga) after each harvest to supply even amounts of N at each application over the year based on an estimated number of harvests for the year (Table 3.1). For example, plantain swards in the 200N treatment were predicted to be harvested ten times in the year so received an application of 20 kg N/ha/y after harvest. Muriate of Potash (50K) was also applied after each harvest to deliver a rate of 100 kg K/ha/y to ensure this nutrient was not limiting.

3.2.3.3 Harvest criteria

The effect of treatments on sward (hereafter termed, forage) DMY was measured in successive harvests from October 2014 to October 2016. Forage-specific criteria were used to decide when harvests were implemented, using current best practice recommendations for each, so that all forages could regrow to their potential and were not constrained by sub-optimal defoliation. The criteria were applied to the 200N sub-plots, as follows: For perennial ryegrass, harvest criteria were met when there were 2.5 leaves/tiller, as per Fulkerson and Donaghy (2001). White clover was harvested when the average sward height was 15 – 25 cm, based on industry recommendations. Chicory and plantain were harvested when average extended leaf length (measured with a ruler) was 35 and 25 mm respectively (Lee et al., 2012). Lucerne was harvested on a 35 – 42-day rotation (Moot, 2001; Teixeira et al., 2007). These variables were monitored once per week during regrowth, in the 200N treatment within each forage main plot, and harvests undertaken when the criteria were met. Once criteria were met, all N treatment sub-plots within each main plot were harvested together. Using this approach, main plot treatments (sward types) were harvested at different points in time, as determined by the rate of the key plant growth processes of each forage.

3.2.4 Herbage sampling

Herbage used to conduct measures of chemical composition and herbage physical properties was collected from plots at the end of one regrowth cycle in February, May, August and November.

Measurements were conducted between 0900 – 1100 h to minimise the effect of diurnal changes on sward characteristics. Herbage was cut from a 5 × 1.5 m long strip (7.5 m^2) within each plot using a Haldrup F-55 forage harvester (Plate 3.2; J. Haldrup a/s, Denmark) set to retain a stubble of 5 cm above ground level. Approximately 4 kg of herbage was sub-sampled from the harvested material, placed in labelled plastic bags and transferred to a refrigerator within 30 mins.

Plate 3.2 Haldrup harvester sampling a lucerne main plot.

The sub-sample was blended in the laboratory and dry matter percentage was determined by drying three sub-samples of 200 ± 5 g in a forced draught oven at 95°C for a minimum of 48 h. The botanical composition of the herbage was determined by hand-sorting a 200 g sub-sample from the bulk sample into leaf, stem, petiole, psuedostem and unsown species. Samples of chicory leaf were further dissected into lamina and midrib (mid-vein or primary vein) Sorted samples were dried in a forced-draught oven at 95°C for 48 h to determine composition on a dry weight basis.

3.3 Herbage biomechanical properties

The biomechanical properties of herbage influence the ease of grazing, and the degree of mastication and rumination required for herbage to be fragmented for swallowing, fermentation, and passage from the rumen (Laredo and Minson, 1975; Vincent, 1983; Wright and Vincent, 1996). Understanding variation in herbage biomechanical properties, and how these may influence cell content release and degradation could better our understanding of feeding to optimise their utilisation. Yet, physical parameters are rarely measured.

There are two important biomechanical properties related to herbivory studies: strength and toughness (Wright and Vincent, 1996). Herbage strength can be defined as the maximum stress at which herbage material breaks, and is a measure of resistance of the material to fracture per unit area (Wright and Vincent, 1996; Sanson et al., 2001). Toughness is often defined as the work (energy) required to fracture the herbage (Perez-Harguindeguy et al., 2013).

Many techniques have been developed and evaluated to measure herbage biomechanical properties (Sanson et al., 2001; Bryant et al., 2008; Perez-Harguindeguy et al., 2013). However, no single method has been widely adopted because the appropriate technique depends on the focus of the research. In pasture-based agriculture, where the focus is the resistance of plant material to shearing (biting off) and comminution of herbage, the three most relevant techniques for measuring herbage biomechanics are the: force to tear, work to shear and force to punch. Reduced leaf shear strength has been related to greater intake rate, enhanced livestock performance (MacKinnon et al., 1988; Inoué et al., 1994) and preference (Bryant et al., 2008) in perennial ryegrass cultivars. Troelsen and Bigsby (1964) showed the degree of comminution of forage by artificial mastication could predict the voluntary feed intake of sheep ($R^2 = 0.94$; $P < 0.01$). The studies reported here measure the force to punch and work to shear rather than force to tear since the focus of the experiments is on the effect of comminution on harvested herbage.

3.3.1 Punch force

Force to punch is a measure of the resistance of herbage tissue to fracture. Punch tests are simple and fast to conduct (Sanson et al., 2001). The force to punch is determined by the circumference of the punch (the length of the shear surface), and the resistance of the material to compression over the area of the punch. Therefore, it is important to note that the measure will vary with different sized punches and care must be taken when comparing studies (Sanson et al., 2001).

Punch force in units of Newtons/mm^2 is given by the equation:

$$Punch\ force = MF/Area$$

Equation 3.1

Where:
MF = maximum force applied (Newtons),
Area = area of the punch (mm^2)

3.3.1.1 Apparatus

The force to punch was measured using a 'punch-and-die' penetrometer (DS2 Digital force gauge, Imada Inc., Japan) (Plate 3.3). The penetrometer was fitted with a flat ended 2 mm diameter punch, giving a punch area of 3.14 mm^2. The punch is attached to a shaft, that is lowered at a constant rate (approximately 1 mm s^{-1}; Aranwela et. al. (1999)) onto a single layer of plant material placed on a die block. A piece of perspex is placed over the material to hold it in place. The perspex and die have a hole in line with the punch when it is lowered which allows the punch to penetrate through the material and into the die. A clearance of 0.05 – 0.10 mm between the side of the punch and the die is necessary to ensure there is no artificial enhancement of material strength (Aranwela et al., 1999) (Figure 3.3). The shaft was connected to a spring-loaded balance and gauge which records the maximum force applied to the material.

Plate 3.3. Punch and die penetrometer (Digital Force Gauge) with a plantain leaf in position on the die block.

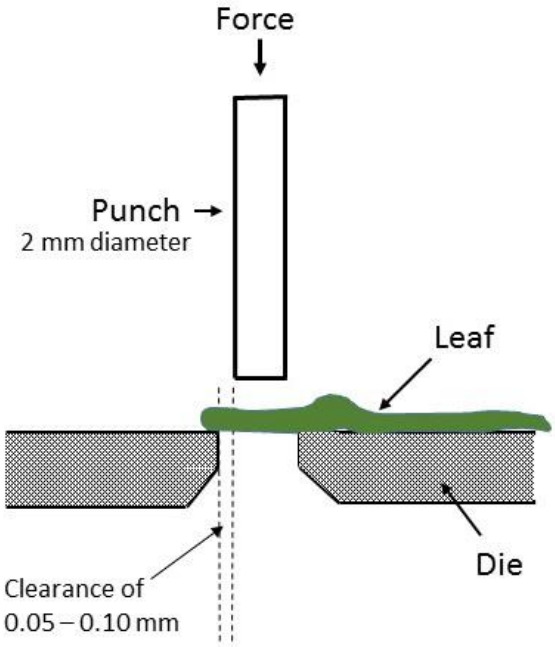


Figure 3.3 Diagram illustrating the configuration for measuring punch force. NB: not to scale.

3.3.1.2 Forage material

Herbage material for punch force measurement was drawn from the bulk sample (section 3.2.4), and stored according to the guidelines suggested by Perez-Harguindeguy et al. (2013). The process was as follows: immediately after harvest, samples were placed in polystyrene bins with ice packs inside then transported to the laboratory for blending and sub-sampling. Sub-samples of approximately 500 g were stored in plastic bags with a wet paper towel, kept in a dark refrigerator (4 °C) and were processed following the punch procedure (section 3.3.1.3) within 48 hours of harvest to minimise water loss via transpiration.

3.3.1.3 Punch procedure

Due to the large variability in anatomy (i.e. tissue arrangement) within stem and leaf tissue (Sanson et al., 2001) the punch size and number of measurements must be carefully considered. A punch size of 2 mm diameter was selected to accommodate the narrow width of perennial ryegrass leaf and to allow a minimum of three measurements per leaf as suggested by Perez-Harguindeguy et al.

(2013) in their guide for standardising methods for measuring plant traits. Where there was sufficient area, 10 punches were made per component (i.e. stem, leaf or petiole) along a transect of the component. For smaller components (i.e. lucerne and white clover leaf or perennial ryegrass psuedostem), three measurements were taken per component, also in transect. For chicory leaf, 10 measurements were also taken along the length of the mid-vein.

A power analysis using a mixed models approach was conducted to determine the number of measurements to be taken per sample. Prior to the experiment (December 2014), test samples of herbage material were collected from the 200N sub-plots in each main plot (five species × 3 replicates). For the test, 50 punch force measurements were taken using leaf tissue from each test sample. Leaf tissue was selected as this was the most prevalent tissue type in the herbage across all species. Using a desired power level of 80% to detect significance at a 95% level of confidence with a CV less than 10%, analysis showed a within plot variance of 0.0234 and between species variance of 0.388 Newtons. The CV declined with increasing numbers of measurements, but only small improvements were achieved by increasing the number of measurements beyond 50 per sample (Table 3.2). This was due to variation among replicates, which could only be overcome with a greater number of replicates, which was not possible. However, results from the preliminary study did show significant differences between forage when 50 measurements were taken, therefore 50 measurements were made per sample.

Randomly selected pieces of the herbage were taken until 50 measurements were recorded for each component (stem, leaf etc.). The mean punch force was calculated for each component, and the weighted average punch force of the forage was calculated by multiplying the average force to punch each component by its proportion in the sward, using the botanical composition data (detailed in Section 3.2.4).

Table 3.2 Results of the power analysis calculation describing the effect of measurement number on the standard deviation (SD) and coefficient of variation (CV) of force to punch (Newtons).

No. of measurements	SD	CV%	Comment
1	0.6414	28.61	
4	0.3470	15.48	
10	0.2494	11.12	
50	0.1765	7.87	80% power of detecting a 25% difference between species
200	0.1592	7.10	80% power of detecting a 20% difference
10000	0.1531	6.83	80% power of detecting a 19% difference

3.3.2 Energy required for maceration

Few studies have investigated the toughness of herbage, but toughness can be estimated by measuring the amount of energy or 'work' required to fracture the material at a stable velocity (Lucas and Pereira, 1990; Vincent, 1990; Aranwela et al., 1999). Chenost (1966) developed a method to measure the energy required to pulverize 25 varieties of hay through a laboratory mill fitted with a 1 mm screen. A 'fibrousness index' derived from this correlated closely with the digestibility ($R^2 = 0.931$) and voluntary feed intake ($R^2 = 0.896$) of the hays, suggesting that feeding value could be predicted from a measure of the toughness of feed material.

Because the focus of the research undertaken here was on fresh herbage, Cheost's (1996) methodology was considered unsuitable and a modification of the mincing technique used for preparing herbage for use in *in sacco* incubation studies of feed (Waghorn and Caradus, 1994), was used. In brief, frozen chopped herbage was fed into mincer with large (12 mm) holes in the sieve plate at a consistent rate to generate material with a particle size distribution similar to that in the rumen of sheep fed herbage. The technique has also been used in studies of feed digestibility in cattle (Burke et al., 2000; Chaves, 2003; Chaves et al., 2006; Sun et al., 2010; Minneé et al., 2017). Preliminary investigation of the energy required to macerate herbage reported by Waghorn et al. (2007) from digestibility studies by Burke et al. (2000) revealed a close correlation between energy required for mincing (J/g DM) and forage NDF concentration ($R^2 = 0.82$). The protocol developed for this study built upon Waghorn et al. (2007).

3.3.2.1 Apparatus

The amount of energy required to mechanically macerate herbage with a Kreft Compact mincer (Kreft, Germany) was measured by a HOBO® analogue logger (Onset Computer Corporation, MA, USA) which recorded AC current (amps) at 1 s intervals (Plate 3.4). Data were downloaded from the logger to a laptop running the HOBOware software package.

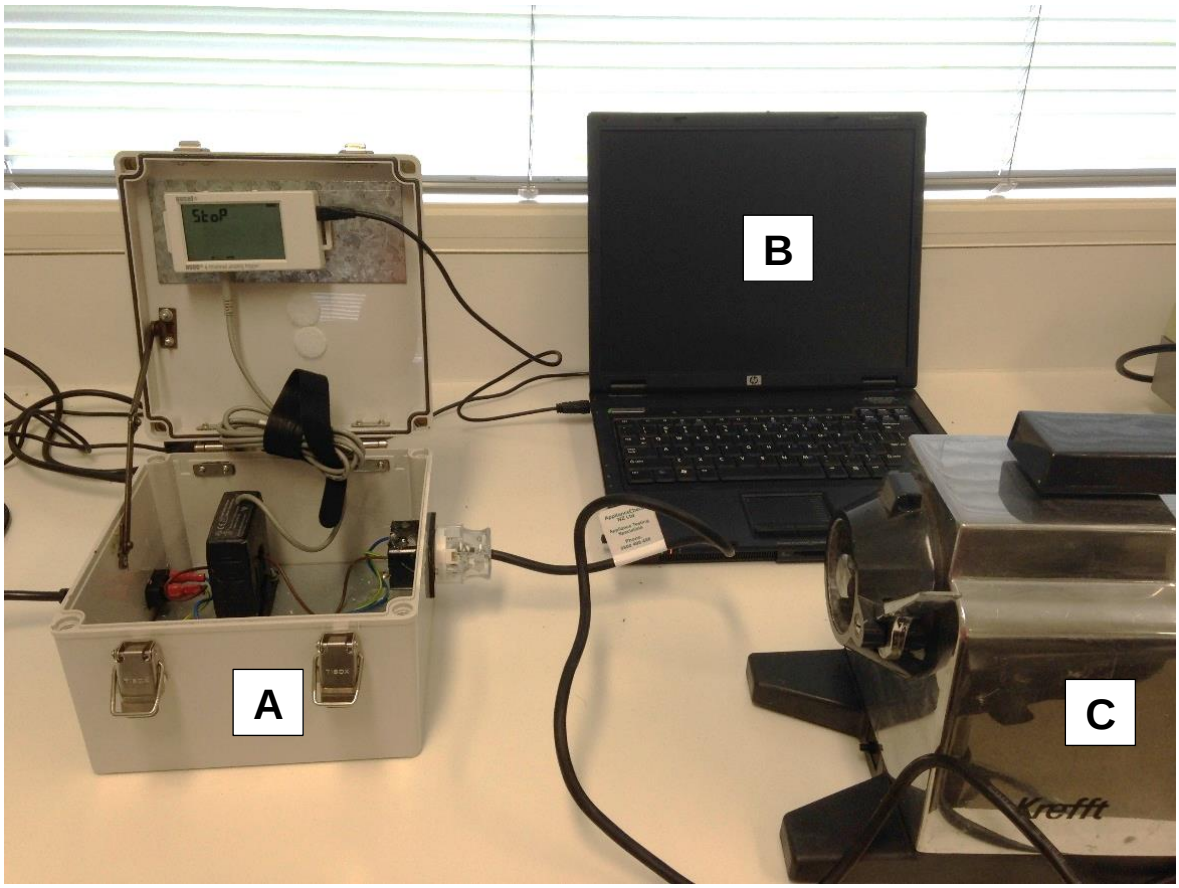


Plate 3.4 Arrangement of the logger (A), laptop (B) and mincer (C). NB: mincing head not attached.

To prevent the herbage material from thawing, which results in excessive crushing rather than cutting, the head of the mincer was modified to keep it cold. An ice-box was built to fit around the mincer head and filled with a mixture of dry and crushed ice (Plate 3.5). A tray from which material was fed into the mincer was inserted into the top of the mincer, and a tray to collect minced samples was placed on scales under the mincing head.

Plate 3.5 Mincer fitted with mincing head (containing screw, cutter blades and sieve plate), surrounded by ice box, and sample tray.

3.3.2.2 Forage material

Approximately 2 kg of fresh herbage was sub-sampled from the blended, bulk sample (section 3.2.4) and frozen (- 20 °C) within 30 minutes of harvest. Frozen samples were chopped into about 20 mm lengths by guillotine to facilitate feeding into the mincer (Burke, 2004). Chopped herbage was kept frozen during chopping and mincing.

3.3.2.3 Method development

The detailed Standard Operating Procedure for the maceration method is presented in Appendix A.1. Since the method was new, it was necessary to determine if results were influenced by the duration of operation, repeatability and to develop methods to relate power consumption to the energy required to macerate material. Some details are provided below.

3.3.2.3.1 Cold start test

The first objective was to determine if leaving the machine idle for several minutes between samples would affect the amount of amps the mincer was drawing (base current) irrespective of sample characteristics. To test this, the current drawn was monitored continuously for 180 s after the

machine was turned on for the first time (cold start) and when it was re-started after intervals of 1, 2, 4, 8, 12 or 24 minutes since the preceding 180 s of operation. The results are illustrated in Figure 3.4 which shows that the amount of current used when the mincer is first turned on (time = 0 min) is considerably greater than when the mincer had been running previously. Therefore, the protocol stipulated that the mincer should be run (or 'warmed up') for a minimum of two minutes before sample measurement commenced, but that the mincer could be run after 2 – 24 minutes of being idle and this would not affect the base current.

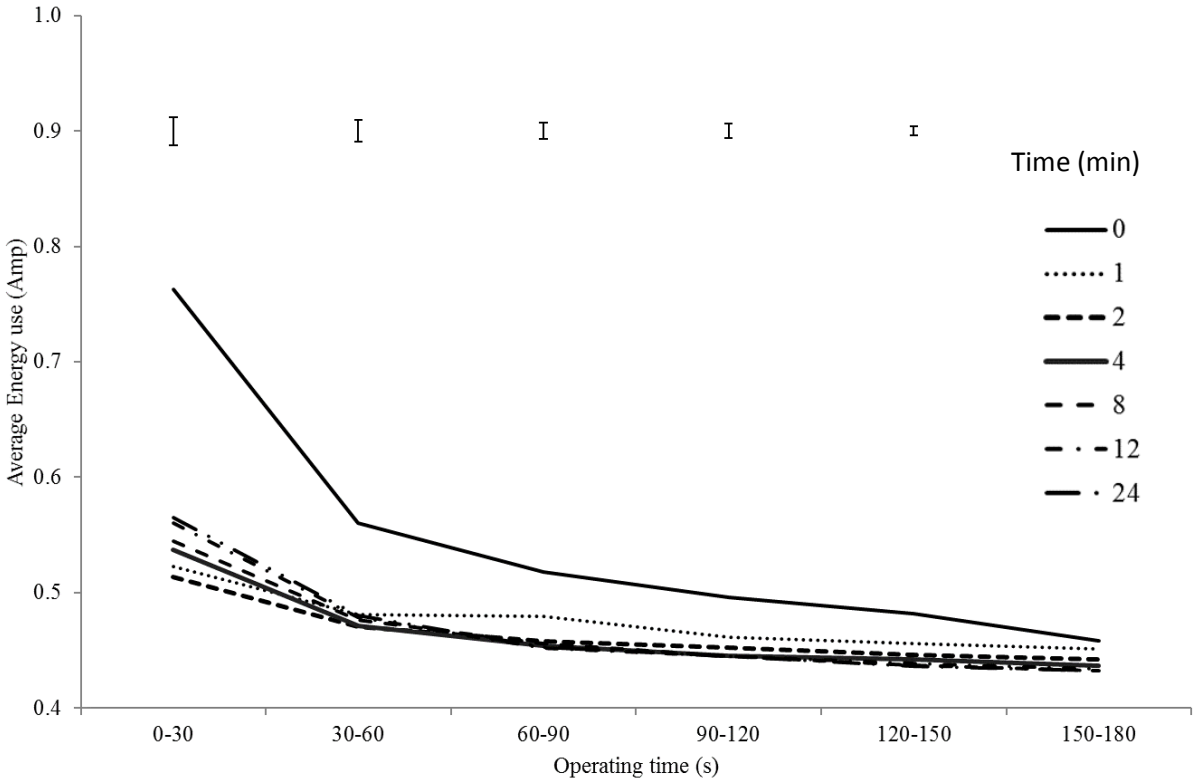


Figure 3.4 Cold start test of mincer. Legend details whether the machine was started cold (time = 0) or the number of minutes lapsed between stopping the mincer and restarting to measure (i.e. Time = 1, 2, 4 …24 minutes). Bars represent standard error.

The data from the cold start test also showed that when the mincer is first turned on it will draw a peak of current and then settle to a constant rate of consumption. Plate 3.6 shows that after the first time the machine is turned on, the time the machine takes to settle to a constant rate of current use is reduced to less than 30 seconds. Therefore, it was determined that the mincer ought to be run for 30 seconds before commencement of measuring the energy required to mince a sample.

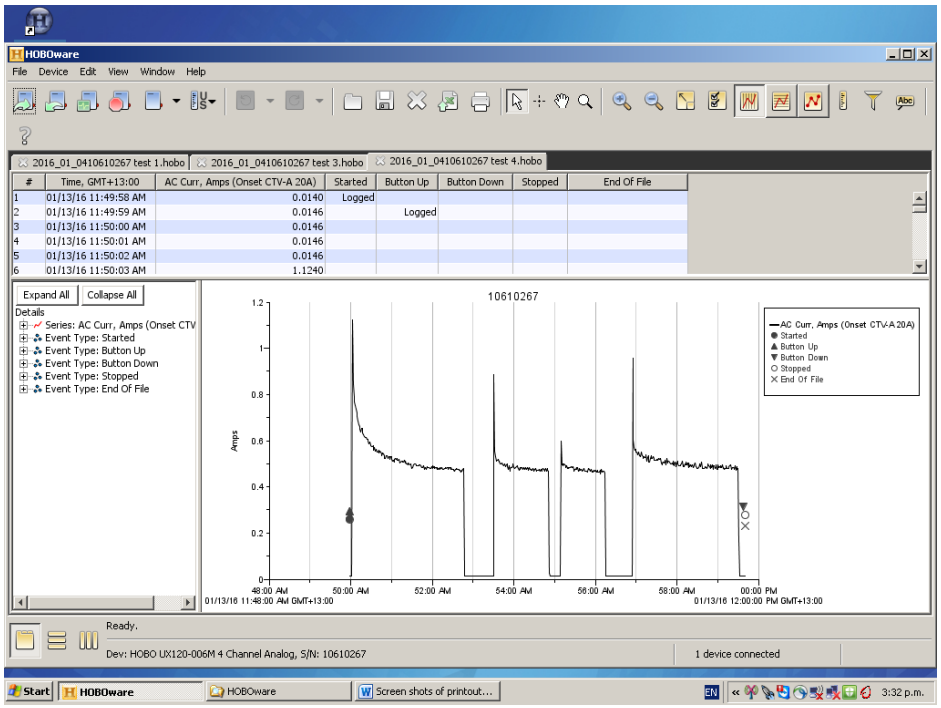


Plate 3.6 Graphical output of logged current showing the initial peak of current consumption at start, then the decline in energy consumption over time. The four peaks show the four times the mincer was turned on during the test period.

3.3.2.3.2 Sample base load determination

Development of the methodology suggested that herbage from different forages had different base loads (Table 3.3). When a sample of the herbage is fed into the mincer, the base load is the current the mincer is consuming when the mincer head is full and the mincer is turned on, but no more material is being fed in, likely due to friction caused by the material in the mincer head against the rotating screw that feeds the chopped herbage forward to the cutter and sieve plate. Therefore, it was determined that a baseload measurement should be taken at the start and end of every batch of samples measured, and each batch would contain like samples. Minced herbage will only flow from the mincer when chopped herbage is fed into the mincer, and the mincer contains approximately 50 g of wet herbage. To calculate baseload, a sample of forage material was fed into the mincer to fill the mincer head. Once full (i.e. some had exited through the sieve plate), no more material is fed into the mincer and the current required to run the machine is measured over 120 s to obtain an average base load current consumption.

Table 3.3 Mean baseload for each forage minced.

Forage	Mean baseload (AMP/s)	SE
Perennial ryegrass	0.80	0.022
White clover	0.61	0.011
Lucerne	0.75	0.018
Chicory	0.65	0.021
Plantain	0.57	0.012

SE = Standard error

3.3.2.3.3 Measuring energy for maceration

The standard protocol adopted for measuring energy consumption during maceration stipulated that, once the base load measure is taken, approximately 75 g of fresh material is fed into the mincer to 'purge' any residual material from the mincing head. Then approximately 700 g of sample material is fed into the mincer at a consistent rate and the energy consumed (amp/s) is recorded by the HOBO logger. The fresh weight (g) of the material discharged from the mincer is recorded, and frozen for further analyses. This was repeated with another two samples, with purges between each sample, and a base load measure taken at the end of the third sample. A second batch of three samples was minced, then the data for all six samples were downloaded into an excel spreadsheet.

The amount of energy required to mince (e.g. Table 3.5) was calculated from the equation:

$$\textit{Current to mince forage (amp/s)} = \textit{current energy (amp/s)} - \textit{baseload current (amp/s)}$$

Equation 3.2

Table 3.4 Logger output data, with mean current and baseload enabling calculation of net energy requirement/second.

Date	Batch	#	Date Time, GMT+12:00	Time	Sample ID	Total current used (amp/s)	Base load current (amp/s)	Current to mince forage (amp/s)
23/06/2016	3	2678	06/23/16 12:35:33 PM	12:35:33	8. NA.103	1.5085	0.57	0.9385
23/06/2016	3	2679	06/23/16 12:35:34 PM	12:35:34	8. NA.103	1.1518	0.57	0.5818
23/06/2016	3	2680	06/23/16 12:35:35 PM	12:35:35	8. NA.103	1.5702	0.57	1.0002
23/06/2016	3	2681	06/23/16 12:35:36 PM	12:35:36	8. NA.103	1.8585	0.57	1.2885
23/06/2016	3	2682	06/23/16 12:35:37 PM	12:35:37	8. NA.103	2.0014	0.57	1.4314

The total current consumed in mincing each sample was calculated on a wet weight (WW) and dry weight (DW) basis from the equations:

$$Amp/g\ WW = WW / SA$$

Equation 3.3

$$Amp/g\ DW = SA / ((WW / 100) \times DM)$$

Equation 3.4

Where:

SA = Sum of amp/seconds used to mince
WW = Wet weight of sample minced (g)
DM = DM % of the sample (measured as described in section 3.2.4)

Total energy consumed per unit wet or dry weight (e.g. Table 3.5) was derived as follows:

$$Energy\ in\ Watts = Amps \times Volts\ (V = 240)$$

Equation 3.5

Where: 1 Watt/s = 1 Joule/s

Table 3.5 Example of calculations to determine energy used to mince fresh herbage, expressed in terms of fresh or dry weight of sample.

Sample ID	Date collected	Expt	Rep	Species	N fertiliser rate (kg/ha)	Sum of AMP	Sample WW (g)	AMP/g WW	Watt or Joule/ g WW	DM%	Sample DW (g)	AMP/g DW	Watt or Joule/ g DW
2421.NA.013	20-Apr-15	N rate	1	WCL	0	271	1435	0.189	45.4	14.6	210	1.293	310
2421.NA.014	20-Apr-15	N rate	2	WCL	0	170	913	0.186	44.6	14.6	133	1.275	306

3.3.2.3.4 Repeatability and sensitivity

Repeatability of the method was determined by mincing three sub-samples from each of four forage species (Table 3.6). The coefficient of variation was less than 10% for all forages, indicating an acceptable level of repeatability.

Table 3.6 Mean energy (amp/s) to macerate three sub-samples of herbage from four forage species.

	Sub-sample						
Forage	1	2	3	Mean	SD	SE	CV%
Chicory	0.4688	0.4554	0.4604	0.4615	0.0055	0.003	1.20
Plantain	1.1239	0.9627	1.0825	1.0564	0.0684	0.039	6.47
Perennial ryegrass	2.3645	2.7729	2.5756	2.5710	0.1668	0.096	6.49
White clover	0.5029	0.4928	0.4863	0.4940	0.0069	0.04	1.39

The sensitivity for detecting differences in mincing energy requirements between forages was assessed at the first sampling (autumn 2015). The mincing energy measure detected differences ($P < 0.05$) of 11.6% or > 0.032 amps/g WW between the five forages with three replicates per forage (Table 3.7).

Table 3.7 Mincing energy required to macerate herbage material (J/g WW) of five forage species.

Forage	Mincing energy
Chicory	26.4^{e}
Lucerne	69.6^{b}
Plantain	48.0^{c}
Perennial ryegrass	151.2^{a}
White clover	36.0^{d}
Mean	67.2
SED	7.2

SED, Standard Error of the Difference
Columns with different superscripts are significantly different ($P < 0.05$)

4

The physical and chemical characteristics of forage species, and the comminution of herbage and release of nutrients during maceration.

4.1 Introduction

The feed value (FV) of a forage species can be defined as the livestock production response to herbage consumed, and is a function of the nutritive value (NV) multiplied by the potential intake of that forage (Ulyatt, 1981). The NV of herbage is a measure of how well it meets animal requirements for nutrients and energy, and is often indicated by chemical composition of the herbage. Whilst the chemical composition (combined with the level of feed intake) determines the upper limit of how much nutrient an animal will receive, there is less knowledge of how those nutrients are released from the herbage and made available to the animal. The implications of variations in rate and extent of nutrient release relate to how this influences utilisation of nutrients for product and in minimising waste. In temperate grazing systems, the concentration of the macronutrient nitrogen (N) in herbage often exceeds livestock requirements. Excess dietary N is degraded in the rumen, absorbed into the blood and excreted in the urine, becoming a source of environmental pollution (Ledgard et al., 1999a). Research into plant × animal interactions that could help identify points for minimising N excretion is essential for reducing the environmental footprint of forage based livestock production systems.

Waghorn and Clark (2004) reported between 50 and 80% of plant cells are ruptured during mastication. Since all non-fibre carbohydrates and most nitrogenous compounds of plants are stored within the cell (Sanderson and Wedin, 1989; Acosta et al., 2007), this means a considerable proportion of the degradable nutrients are released during ingestive mastication. Importantly, Burke (2004) and Minneé et al. (2017) showed that the amount of N (as crude protein, CP) released during mechanical comminution of feed material differed between forage species (forages). However,

none of these studies sought to determine the characteristics of herbage that influenced rupture and release. Quantifying the variation in nutrient release between forages could aid our understanding of herbage FV, or explain variations in nutrient use efficiency (NUE) and N excretion between forages (i.e. Woodward et al. (2012) Minneé et al. (2017)).

Furthermore, the physical attributes of forages, such as their biomechanical properties, are rarely considered (Wheeler and Corbett, 1989; Smith et al., 1997). This is so, despite agreement that these properties influence palatability, grazing, and the amount of time spent orally processing feed by livestock, which ultimately affects dietary intake and thus FV (MacKinnon et al., 1988; Inoué et al., 1994; Van Soest, 1994). The paucity of data may be due to a lack of routine laboratory methods for assessing the resistance of herbage material to breakdown. The experiment detailed in this chapter employs a novel laboratory method for determining the physical resistance or 'toughness' of herbage (detailed in Chapter 3, section 3.3.2 Energy required for maceration) and examines relationships between herbage toughness, cell rupture and the release of nutrients during maceration.

The aim of this experiment was to quantify the variation in N release from five common forage species (ryegrass, white clover, lucerne, chicory, and plantain), and to identify the characteristics that influence herbage comminution and release of nutrients. The hypothesis is that relationships exist between species' physical and chemical characteristics, and the extent of comminution and N released during maceration.

4.2 Materials and Methods

4.2.1 Herbage sampling

Details of sward establishment and management are given in sections 3.2.1 and 3.2.3. Briefly, replicated (n = 3) monoculture swards of five forage species were sown in a randomised split-plot design (plot size 9 x 6 m) in October 2014 at DairyNZ's Scott Farm property, near Hamilton, New Zealand (37°47′S, 75°19′E; 40 m.a.s.l) on a Horotiu silt loam. They were: perennial ryegrass, white clover, lucerne, chicory, and plantain.

The herbage material used in this study was obtained from harvests of plots receiving 200 kg N/ha/y, corresponding with the last month of each season: February (summer), May (autumn), August (winter) and November (spring) during the 2015 year. Collection occurred when each forage reached target harvest criteria (described in Section 3.2.3) and because the forages differed in growth rate, they were not necessarily on the same date. Harvest was conducted between 0900 and 1100 h to minimise variation in chemical composition and turgor pressure in the material. Once sampling was complete, samples were stored in a refrigerator (4°C) until processing was complete.

4.2.2 Herbage physical characterisation

Herbage DM content was determined by weighing and oven-drying three subsamples of 200 ± 5 g wet weight at 95°C for 48 h.

Botanical composition of the herbage was determined by hand sorting sub-samples (200 g) into green leaf, dead leaf, stem, petiole, and unsown species. Sorted samples were dried in a forced-draught oven at 95°C for 48 h to determine botanical composition on a dry weight basis.

For morphological measurement, from each sample, 100 pieces were randomly sampled and the length of the stem, and the length and width leaf blade were measured using a standard ruler or digital callipers where the leaf or stem was less than 20 mm in size (Mitutoyo digimatic CD-8"CS, Mitutoyo Corp, Japan).

Leaf and stem strength was described by measuring the force (Newtons) required to punch a 2 mm diameter hole in the material using a digital force gauge (DS2 Digital force gauge, Imada Inc., Japan). Detailed methodology is given in Chapter 3 (section 3.3.1). In brief, for each sample 50 punch force measurements were taken for each component (leaf, stem and petiole) present in the sample. The physical strength of the herbage was estimated by multiplying the average force required to punch each component by the percentage of that component in the herbage, as determined by the botanical composition measurement.

The relative toughness of the herbage was measured by determining the amount of energy required to mechanically macerate the forage material using a Kreft Compact mincer (Kreft, Germany) (methodology detailed in Chapter 3, section 3.3.2). Macerated material was collected and frozen, for evaluation of the extent of comminution (particle size distribution) and nutrient release by maceration (section 4.2.4).

4.2.3 Herbage chemical characterisation

A 200 g sub-sample was frozen at -20°C, freeze dried and ground to pass through a 1 mm sieve (Christy & Norris Mill, United Kingdom) for chemical analysis. Structural carbohydrates, neutral detergent fibre (NDF) was measured using the methods of Van Soest et al., (1991), and acid detergent fibre (ADF) by method 973.18 (AOAC Int. 2000), using Whatman 934-AH glass micro-fibre filters with a 1.5 µm particle retention. Fibre recovered from the ADF measurement was washed in sulphuric acid, then dried and ashed for 2 h to determine lignin content as per Goering and Van Soest (1970). Water soluble carbohydrate content (WSC) was measured colorimetrically with sucrose as a standard, by the method of DuBois et al. (1956). Total N concentration was determined by combustion (method 990.03; AOAC Int. 2000) in a LECO FP-528 Nitrogen analyser (Leco, St Joseph, MI, USA). Nitrogen in the NDF residue (neutral detergent insoluble N, NDIN) was measured in the residue from the NDF procedure also using the LECO FP-528 analyser. Concentration of total non-protein N components was assessed by the urease method (941.04; AOAC Int. 2000) and nitrate by the potentiometric method (986.31; AOAC Int. 2000). Total ash concentration was determined by heating 1.5 g of sample to 600°C in porcelain crucibles for 4 h (method 942.05; AOAC Int. 2000).

4.2.4 Herbage comminution and cell content release

The effect of maceration on the physical breakdown (comminution) of the herbage was determined by evaluating the particle size distribution (PSD) of the macerated herbage using a wet sieving apparatus (Turner and Newell Ltd) with four counter-rotating sieves. Sieve sizes (as the length of the size of the square hole) were 4, 2, 1, 0.5 and 0.075 mm. Duplicate subsamples of 25 g (fresh weight) were placed on the top sieve, then 1500 ml of water recirculated through all sieves at a flow

rate of 4 litres/minute for 5 minutes. Material retained on each sieve was transferred on to weighed filter papers then oven-dried at 60 ºC for 48 h before weighing to determine PSD on a DM basis. The soluble fraction was defined as the DM passing the 0.075 mm sieve, and was calculated as the difference between the total DM sieved and the sum of the DM retained on the five sieves.

For determination of nutrient release from maceration, 100 g sub-samples of macerated herbage were placed in weighed dacron bags (mean pore size 35 µm; Ankom Technology, USA), the bags sealed and then rinsed carefully by hand until no further colour appeared in the rinsing solution. Rinsed samples were freeze-dried, weighed, ground to pass a 1 mm sieve (Christy & Norris Mill, United Kingdom), then analysed for NDF, N and WSC using the methodology described in section 4.2.3. The percentage of N and WSC released was defined as the difference between the quantity of N or WSC in the unmacerated forage material and the quantity in the macerated material divided by the quantity in the unmacerated material. The weight of the sample pre- and post-rinsing was used to correct for loss of DM.

4.2.5 Statistical analyses

Data for each variable were analysed in a repeated measures analysis using a restricted maximum likelihood (REML) approach with mixed models including forage and season as fixed effects, and replicate and plot within replicate as random effects. The data for each season were then analysed using REML including forage as a fixed effect. When a significant forage effect was observed, multiple comparisons were made using Fishers protected least significant difference test.

Relationships between variables were investigated using regression analysis to fit linear equations, first using the mean values for each forage to test for associations between variables using between forage variation, then using individual replicates to test the association within forage using environmental variation including seasonal variation. The regression analysis provided slope, SE of the slope, *P*-value and the R^2 coefficient. A multiple regression analysis was conducted using an all sub-sets regression to find the best fitting model to predict CP loss and mincing energy. The predictive models were determined using a maximum of five variables. One forage was selected at

random and omitted from the analyses; data from the other four forages were then included to test predictions for the excluded forage. GenStat version 16.2 (VSN International, Hemel Hempstead, UK) was used for all statistical analyses.

4.3 Results

4.3.1 Herbage chemical and physical characteristics

The physical characteristics of the five forages evaluated are presented in Table 4.1 as an average of the four sampling dates, with some individual sampling date data given in Appendix B.1. Dry matter content of the herbage ranged from 8% for chicory in autumn to 27% for lucerne in summer. Chicory, plantain and perennial ryegrass herbage was predominantly leaf (mean 89%), while lucerne and white clover were approximately half leaf and half stem or stem and petiole. Stem material was only present in spring for chicory, and in spring and summer for plantain, but was present at all sampling dates (seasons) in ryegrass herbage, in increasing amounts as the experiment progressed (0.5, 2, 4 and 18% of the DM in summer, autumn, winter and spring respectively). The forages showed significant variation in stem and leaf length and leaf width ($P < 0.001$, Table 4.1).

There were variations in leaf strength (punch force) detected between forages (appendix B.1, range 0.33 – 1.32 Newtons/mm^2; $P < 0.001$), and stem strength was approximately 3.5 – 10 times that for the leaf in each forage (Table 4.1). Leaf strength was greatest for ryegrass, which required 2 – 4 times more force to punch compared with the other forages evaluated. The weighted strength of the herbage was similar between all forages except lucerne which required approximately twice as much force as the other forages.

Herbage toughness (energy required to macerate) also differed between forage (Table 1; $P < 0.001$). At all sampling dates (appendix B.1), ryegrass herbage required the most energy to macerate ranging from 137 – 192 J/g FW, and chicory herbage usually required the least (3 of 4 sampling dates, range 18 – 47 J/g FW). When expressed on a DM basis, there was a 2-fold range in toughness (277 – 748 J/g DM) but there was no change in the ranking of toughness of the forages studied when expressed on a DM or FW basis.

Table 4.1 Physical characteristics of the five forages. Data are means of the four sampling dates.

	Forage						
	Chicory	Lucerne	Plantain	Perennial ryegrass	White clover	SED	*p*-Value
Dry matter (g/100 g)	10.1^{e}	18.1^{b}	13.4^{d}	21.7^{a}	15.7^{c}	0.9	< 0.001
Composition (%)							
Lamina	96.7^{a}	48.8^{d}	85.6^{b}	83.7^{b}	59.9^{c}	3.8	< 0.001
Stem	2.0^{c}	36.5^{a}	13.0^{b}	6.2^{bc}	6.7^{bc}	2.8	0.003
Petiole	-	8.70	-	-	32.1	-	-
Morphology (mm)							
Lamina width	45.0^{a}	12.4^{d}	28.3^{b}	2.8^{e}	20.3^{c}	0.4	< 0.001
Lamina length	223.1^{a}	22.6^{c}	137.4^{b}	134.8^{b}	22.1^{c}	8.2	< 0.001
Stem length	3.1^{c}	195.1^{a}	107.1^{b}	24.1^{c}	59.8^{bc}	20.6	< 0.001
Petiole length	-	-	-	-	95.7	-	-
Herbage strength [1.] (Newtons/mm^{2})							
Lamina	0.54^{c}	0.41^{d}	0.67^{b}	1.12^{a}	0.55^{c}	0.04	< 0.001
Stem	3.00^{b}	4.17^{a}	2.43^{b}	3.91^{b}	2.45^{b}	0.34	0.002
Petiole	-	-	-	-	2.08	-	-
Weighted whole sward	1.20^{b}	2.16^{a}	0.93^{b}	1.22^{b}	1.21^{b}	0.12	0.001
Herbage toughness[2.]							
J/g fresh weight	29.7^{e}	79.0^{b}	54.2^{c}	162.4^{a}	42.6^{d}	2.9	< 0.001
J/g dry weight	291.5^{c}	441.7^{b}	408.4^{b}	747.9^{a}	276.7^{c}	18.8	< 0.001

[1.] Force to punch
[2.] Energy required to macerate herbage material

The concentration of chemical components is given in Table 4.2. The legumes lucerne and white clover contained the greatest concentration of CP, urea + ammonia N, and nitrate N. The herbs, chicory and plantain, tended to contain the lowest concentration of CP. Nitrogen associated with the fibre (NDIN) was greatest in ryegrass which also contained the most fibre (neutral detergent fibre; NDF) and non-fibre carbohydrates (water soluble carbohydrate; WSC). The WSC assay did not measure all fermentable carbohydrates (beta-glucans, galactans, and pectins), and non-structural carbohydrates (NSC) concentration was calculated by the equation:

$$NSC = 100 - (CP + NDF + Ash + Lipid)$$

Equation 4.1

Table 4.2 Chemical concentration (g/100 g) of herbage from five forages. Data are means of the four sampling dates.

	Forage						
	Chicory	Lucerne	Plantain	Perennial ryegrass	White clover	SED	*p*-Value
Crude protein	14.4[c]	26.9[a]	12.5[d]	16.9[b]	26.9[a]	0.63	< 0.001
Urea + ammonia nitrogen (N)	0.031[bc]	0.043[a]	0.025[c]	0.036[ab]	0.044[a]	0.004	0.004
Nitrate N	0.16[b]	0.48[a]	0.11[c]	0.18[b]	0.46[a]	0.078	0.019
Total Non-protein N[1.]	0.19[b]	0.52[a]	0.12[b]	0.22[b]	0.50[a]	0.089	0.023
Neutral detergent insoluble N	0.28[c]	0.23[d]	0.48[b]	0.64[a]	0.24[d]	0.017	< 0.001
Water-soluble carbohydrates	14.3[c]	9.2[d]	15.7[b]	17.9[a]	12.0[c]	0.60	< 0.001
Neutral detergent fibre	18.3[d]	26.5[b]	24.6[b]	43.0[a]	20.7[c]	0.94	< 0.001
Acid detergent fibre	13.5[c]	21.8[b]	20.6[b]	24.7[a]	14.3[c]	1.00	< 0.001
Lignin	2.8[bc]	4.5[a]	4.9[a]	2.4[c]	3.1[b]	0.18	0.001
Ash	15.7[a]	9.9[c]	14.0[b]	10.2[c]	9.2[d]	0.18	< 0.001
Non-structural carbohydrate[2.]	48.0	38.4	46.6	32.2	39.9		

[1.] Total Non-protein N is calculated as the sum of urea + ammonia N + nitrate N.
[2.] Non-structural carbohydrate (NSC), as calculated by the equation: NSC = 100 – (CP + NDF + Ash + Lipid[3.]).
[3.] Lipid values were obtained from the associated 'Critical N' trial and not reported here.

4.3.2 Characteristics influencing forage biomechanical properties

When all forages were included in the analyses, there was no evidence of a relationship between any of the chemical characteristics measured and the strength of leaf material or the weighted herbage strength (Table 4.3). Within forage, however, there was a positive association between herbage DM content and weighted herbage strength for chicory and lucerne, and between herbage NDF and ADF concentration and weighted herbage strength for all forages except chicory (Table 4.3).

Toughness of herbage was positively correlated with dry matter (DM) content ($R^2 = 0.61$; $P < 0.001$; Figure 4.1a), NDF ($R^2 = 0.88$; $P = 0.005$; Figure 4.1b) and acid detergent fibre concentration (ADF; $R^2 = 0.72$; $P < 0.001$) but not with lignin concentration ($R^2 = 0.02$; $P > 0.5$; Figure 4.1c) when all forages were included in the analyses (Table 4.4). However, the strength of the relationship, varied between forages (Table 4.4). Herbage DM% was only weakly associated with toughness in chicory, but moderately to strongly associated with toughness in the other forages. Similarly, NDF and ADF concentration was most strongly associated with toughness in lucerne but the relationship in other

forages was weak or non-existent. Lignin content explained at least 49% of the variation in toughness in lucerne, plantain and ryegrass but there was no association between lignin and toughness in white clover or chicory material.

Table 4.3 Correlation coefficient (R^2) between herbage strength (Newtons/mm^2) and the dry matter (DM, %) content, neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentration (g/ 100 g DM) of when all five forages were included in the analysis and within individual forages. Figures in bold are significant at the 95% confidence level, and figures in italics are denote a negative relationship.

Forage	DM%	NDF	ADF
All forages	0.13	0.00	**0.08**
Chicory	**0.72**	*0.00*	*0.12*
Lucerne	**0.63**	**0.83**	**0.88**
Plantain	0.03	**0.77**	**0.71**
P. ryegrass	0.02	***0.51***	**0.53**
White clover	**0.38**	**0.50**	***0.42***

Table 4.4 Coefficients for the relationship (R^2) between herbage toughness (maceration energy, J/g fresh weight (FW)) and the dry matter (DM, %) content, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin concentration (g/ 100 g DM) of herbage from five forages individually and the five combined. Figures in bold are significant at the 95% confidence level, and figures in italics are denote a negative relationship.

Forage	DM%	NDF	ADF	Lignin
All forages	**0.80**	**0.99**	0.72	*0.14*
Chicory	**0.38**	*0.07*	*0.24*	*0.01*
Lucerne	**0.61**	**0.68**	**0.73**	**0.83**
Plantain	**0.79**	0.31	**0.43**	**0.49**
P. ryegrass	**0.73**	**0.37**	0.24	**0.62**
White clover	**0.59**	0.03	0.01	0.01

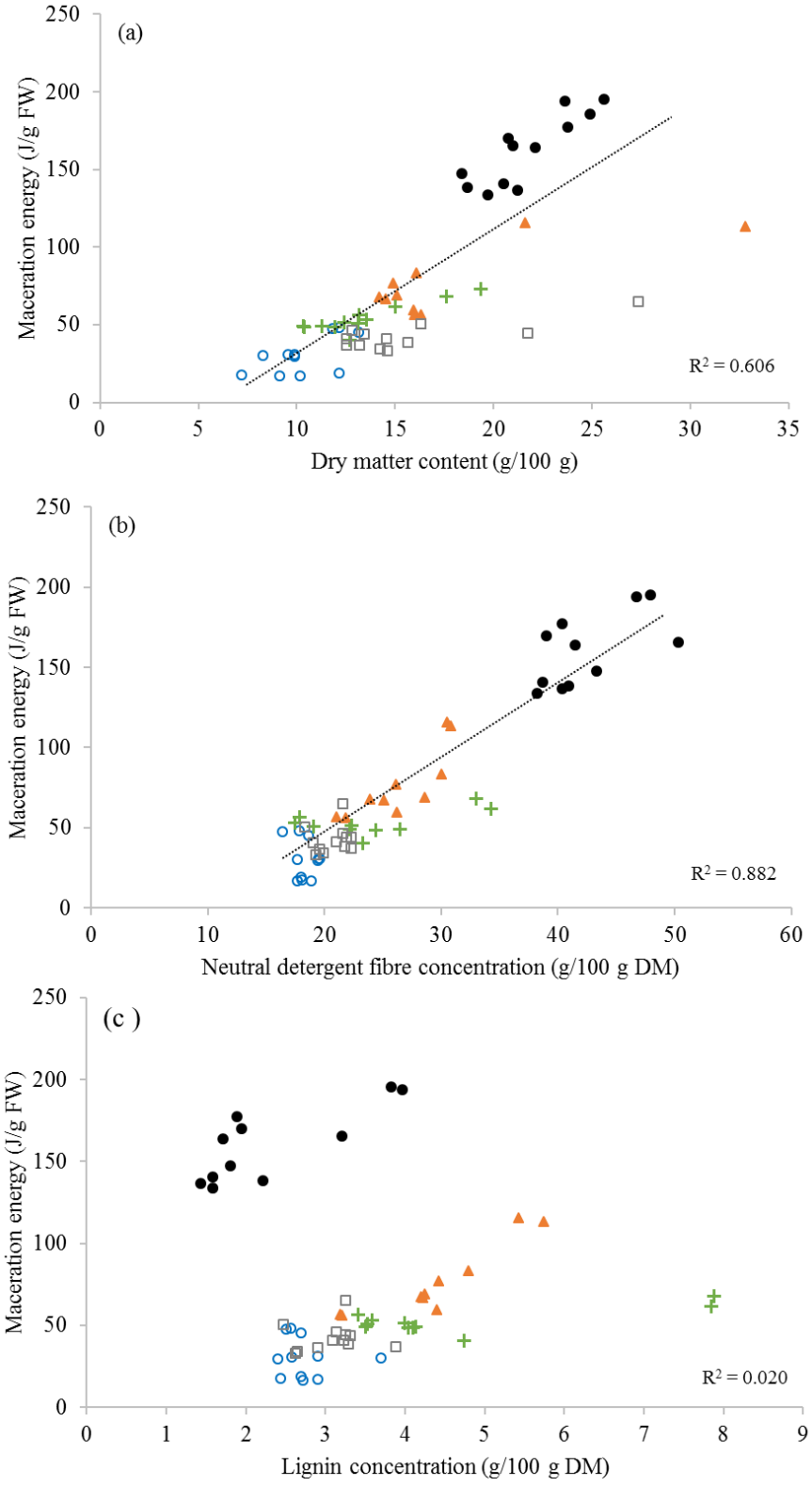


Figure 4.1 The correlation between herbage toughness (maceration energy, J/g fresh weight (FW)) and herbage dry matter content (a), neutral detergent fibre (b), and lignin concentration (c) (g/100 g) of five forages: chicory (○), lucerne (▲), plantain (+), perennial ryegrass (●) and white clover (□).

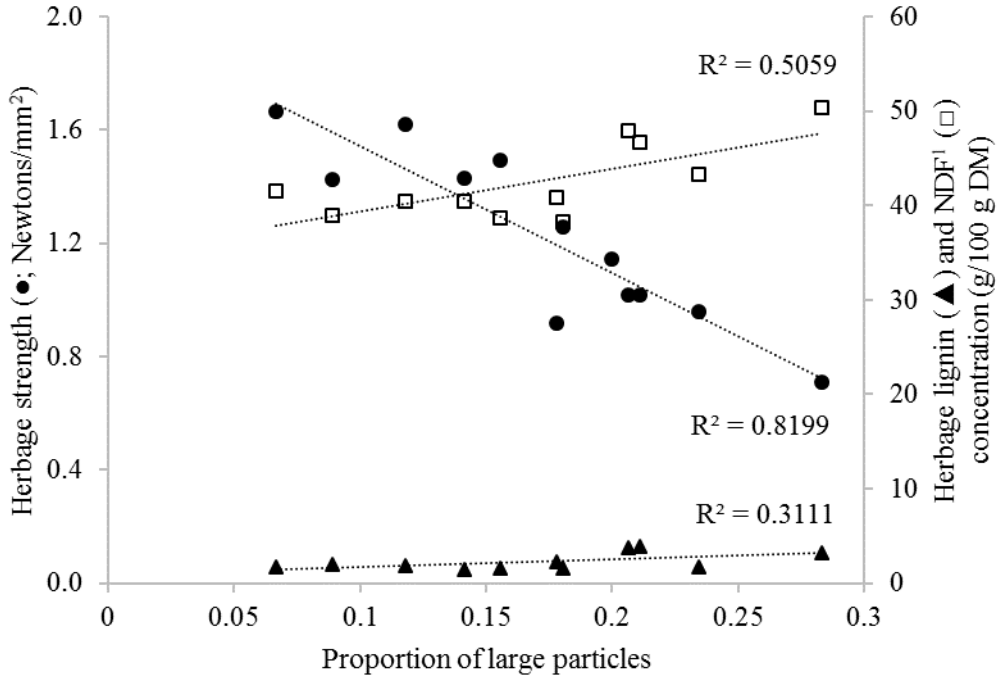
4.3.3 Herbage comminution

The PSD of macerated herbage fragments from the five forages evaluated is summarised in Table 4.5. Differences between forages on individual sieves were minor, and so for clarity PSD was grouped into three categories: 2 + 4 mm, because 2 mm is thought to be the threshold at which particles cannot pass from the rumen of cattle (Poppi et al., 1985); 'soluble' fraction as those particles in suspension that passed the 0.075 mm sieve; and a grouping of the sieves in between, 1 + 0.5 + 0.075 mm. Plantain herbage had the least proportion of large particles (> 2 mm) and the greatest proportion of small particles (0.075 – 1 mm) while the other forages had a similar PSD. The extent of herbage comminution was not significantly related to any of the chemical characteristics at the 5% threshold when all forages were included in the analyses. Within ryegrass herbage, the proportion of large particles in macerated material was negatively correlated with herbage strength ($P < 0.001$), but positively correlated with NDF and lignin concentration ($P < 0.05$) in the herbage (Figure 4.2).

Table 4.5 Distribution of particle fractions (on a DM basis) of macerated material by sieve size from five forages. Data are means of the four sampling dates.

	Forage						
	Chicory	Lucerne	Plantain	Perennial ryegrass	White clover	SED	*p*-Value
Particle size distribution							
4 + 2 mm	0.231^{ab}	0.211^{b}	0.133^{c}	0.248^{ab}	0.269^{a}	0.020	0.002
1 + 0.5 + 0.075 mm	0.372^{c}	0.428^{b}	0.494^{a}	0.373^{c}	0.360^{c}	0.013	< 0.001
Soluble	0.396	0.358	0.373	0.379	0.371	0.019	0.393

Figure 4.2 Relationship between the proportion of large particles in macerated material and herbage strength (Newtons/mm^2), lignin and NDF concentration from perennial ryegrass herbage.



[1] NDF, Neutral detergent fibre.

4.3.4 Crude protein and water-soluble carbohydrate release

The proportion of CP released during maceration ranged from 0.02 – 0.45 (appendix B.2), and differed between each forage in the order lucerne > ryegrass > clover > chicory > plantain (P < 0.001; Figure 4.3 a). For most species, release of CP was unaffected by season except for chicory where the proportion of CP released ranged from 0.08 in summer and winter to 0.22 and 0.34 in spring and autumn respectively (P forage × season = 0.002; appendix B.4). Proportional release of WSC was on average 3.5 times greater than that of CP (Figure 4.3), with at least 0.85 of WSC released during maceration from all forages. Proportionately, WSC release was greater in herbs compared with the other species (P = 0.019).

When the amount (g/100 g DM) of CP released was determined, the ranking of CP release was similar to the ranking of proportional CP release, with lucerne releasing the most (11.5 g/100 g DM), which was 15 times more than plantain that released the least CP at 0.8 g/100 g DM (Figure. 4.3 b). The amount of WSC released was more variable than proportional release, ranging from 8.0

– 15.7 g/100 g DM. The amount of WSC released exceed that of CP release in all forages except lucerne.

No single chemical characteristic was associated with the release of CP (Table 4.6) or WSC (Table 4.7) from all forages during maceration. Forages that tended to lose the greatest proportion of CP also tended to contain the greatest concentration of CP in the herbage, but the relationship was weak and not significant when all forages were included in the analysis (Table 4.6, Figure 4.4; $R^2 = 0.44$; $P = 0.22$). However, for some forages the amount of CP released was highly correlated with CP concentration, e.g. in chicory ($R^2 = 0.89$; $P < 0.001$). Across all species, herbage DM content was related ($P < 0.05$) to the amount of WSC ($R^2 = 0.75$) and CP released ($R^2 = 0.58$), but within forages the relationships varied from positive to negative, and from non-existent to strong (i.e. white clover $R^2 = 0.75$) (Table 4.6, Table 4.7, Figure 4.5).

Similarly, there were no physical characteristics (e.g. morphology, biomechanical properties etc.) associated with release of CP or WSC common to all species. Herbage toughness did not affect CP release, although 68% of the variation in CP release was related to herbage strength when all forages were included in the analysis. But the relationship was strongly affected by a positive relationship in white clover and a negative relationship in chicory herbage i.e. that as chicory herbage strength increased, CP release declined (Table 4.6). Loss of WSC was weakly correlated with lamina strength in lucerne, plantain and clover ($R^2 = 0.40$, $P < 0.05$) (Table 4.7). The extent of forage comminution (indicated by the PSD) accounted for 30 - 40% of variation in CP and WSC release (Tables 4.6 and 4.7).

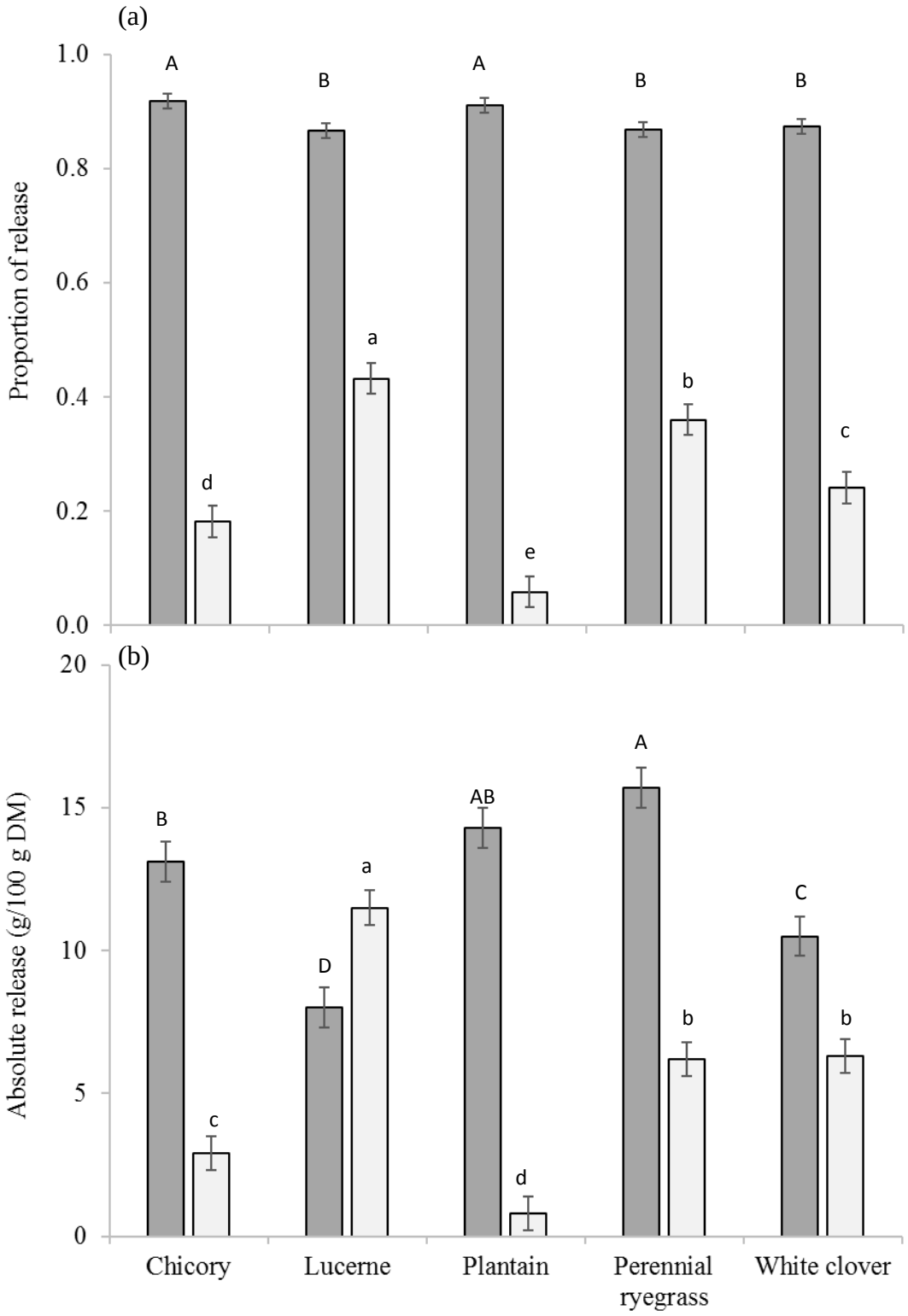


Figure 4.3 Average proportion (a) and amount (g/100 g DM) (b) of water soluble carbohydrate (dark grey bars) and crude protein released (light grey bars) after maceration of five species. Note: bars sharing the same letter in the same case are not statistically different.

Table 4.6 Coefficients for the relationship (R^2) between herbage physical and chemical characteristics and crude protein release during maceration of herbage. Figures in bold are significant at the 95% confidence level. Figures in italics denote a negative relationship.

	Crude protein release					
	All forages	Chicory	Lucerne	Plantain	P. ryegrass	White clover
DM (%)	**0.58**	***0.55***	**0.42**	*0.04*	0.06	**0.75**
Leaf content (%)	*0.41*	*0.07*	*0.03*	*0.03*	0.13	*0.16*
Leaf width (mm)	*0.52*	**0.45**	*0.11*	0.12	0.05	*0.01*
Leaf strength (Newtons/mm^2)	0.00	***0.71***	0.13	*0.05*	*0.02*	**0.46**
Weighted sward strength (Newtons/mm^2)	0.68	***0.43***	0.07	0.02	0.03	**0.44**
Herbage toughness (J/g FW)	0.32	*0.09*	0.07	*0.02*	0.09	**0.56**
Large particles (%)	0.37	*0.00*	***0.51***	0.13	*0.05*	0.03
Soluble particles (%)	0.30	0.01	0.15	0.27	0.26	*0.04*
Crude protein (g/100 g)	0.44	**0.89**	*0.08*	0.15	0.36	*0.26*
NPN (g/100g)	**0.37**	**0.23**	*0.01*	0.00	0.03	*0.53*
WSC (g/100 g)	*0.13*	*0.08*	0.16	*0.20*	*0.01*	0.14
NDF (g/100 g)	0.25	0.09	0.07	0.09	*0.02*	0.18
ADF (g/100 g)	0.25	0.21	0.03	0.02	*0.06*	0.07
Lignin (g/100 g)	*0.03*	0.01	0.09	0.03	0.02	0.17

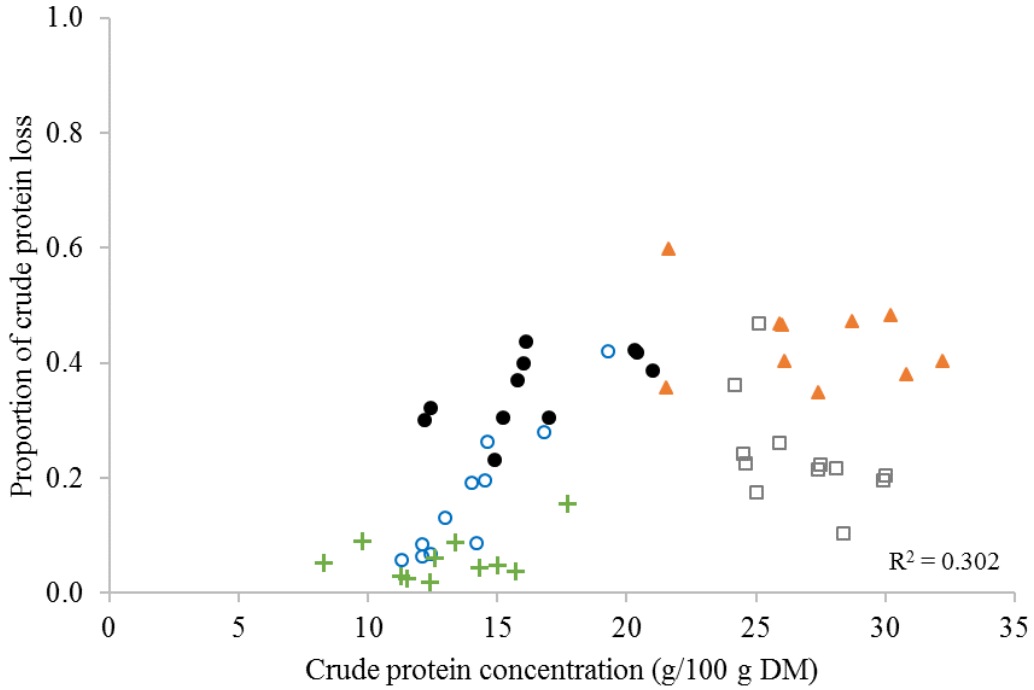


Figure 4.4 The relationship between the proportion of crude protein release and herbage crude protein concentration (g/100 g) of five forages: chicory (○), lucerne (▲), plantain (+), perennial ryegrass (●) and white clover (□).

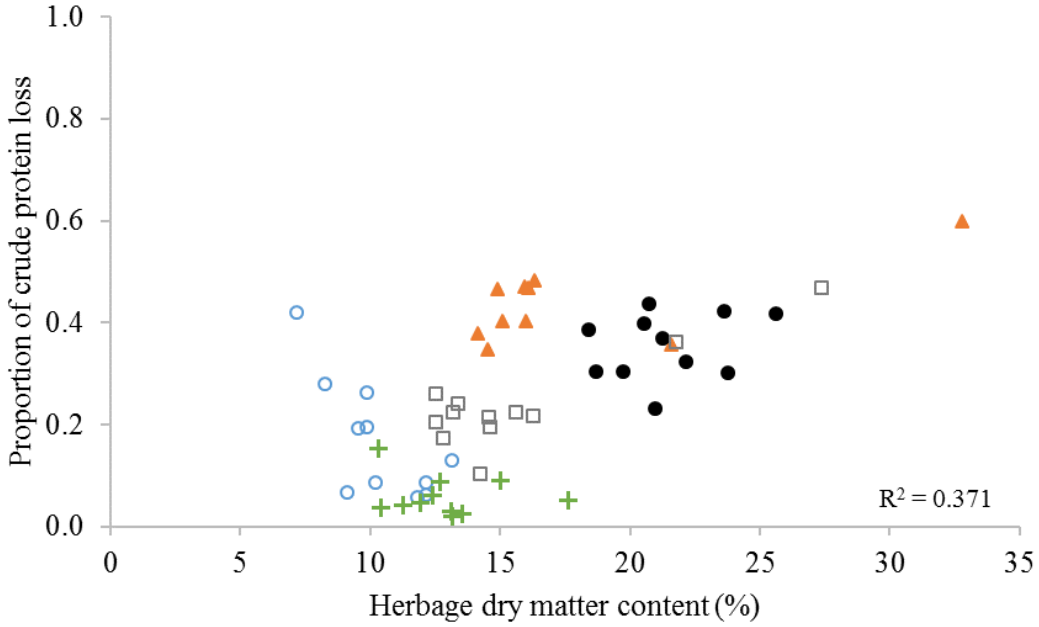


Figure 4.5 The relationship between the proportion of crude protein loss and herbage dry matter content (%) of five forages: chicory (○), lucerne (▲), plantain (+), perennial ryegrass (●) and white clover (□).

Table 4.7 Coefficients for the relationship (R^2) between herbage physical and chemical characteristics and water soluble carbohydrate release during maceration of herbage. Figures in bold are significant at the 95% confidence level.

	Water soluble carbohydrate release					
	All forages	Chicory	Lucerne	Plantain	P. ryegrass	White clover
DM (%)	0.75	0.27	0.08	0.06	**0.39**	**0.39**
Leaf content (%)	0.54	0.09	0.00	**0.46**	0.12	0.11
Leaf width (mm)	0.76	0.23	0.39	0.08	**0.40**	0.02
Leaf strength (Newtons/mm^2)	0.03	0.2	**0.43**	**0.41**	0.16	**0.40**
Weighted sward strength (Newtons/mm^2)	0.24	0.07	0.00	0.21	0.19	**0.37**
Herbage toughness (J/g FW)	0.31	0.10	0.01	0.01	**0.56**	0.17
Large particles (%)	0.34	0.09	0.34	0.28	0.15	0.02
Soluble particles (%)	0.45	0.23	**0.48**	**0.38**	0.28	0.01
Crude protein (g/100 g)	0.60	0.01	0.04	0.13	0.22	0.33
WSC (g/100 g)	0.07	0.13	**0.59**	**0.47**	**0.65**	0.12
NDF (g/100 g)	0.27	0.04	0.01	0.14	**0.49**	0.27
ADF (g/100 g)	0.18	0.04	0.01	0.09	**0.39**	0.08
Lignin (g/100 g)	0.04	0.01	0.01	0.03	**0.79**	0.18

4.3.5 Predicting crude protein release and herbage toughness

The variables with the strongest correlations were selected for the multiple-regression analyses to predict CP release and herbage toughness. These were DM%, CP concentration, leaf punch force, leaf width and weighted sward strength for CP loss; and DM%, NDF concentration, ADF concentration, lignin concentration and stem % for predicting maceration energy (toughness). Ryegrass was selected as the forage to be omitted. None of the fitted models using one to five variables predicted CP loss or maceration energy from ryegrass well. The model explained 74% of the variation in CP loss for chicory, plantain, lucerne, and white clover (SE = 8.18) but the fit was poor for ryegrass.

4.4 Discussion

Fibre and water soluble carbohydrate concentrations of the species evaluated in this study were within the range reported from the same species grown in the same geographic region (Waikato, New Zealand) or at a similar growth stage (Harris and Clark, 1996; Harris et al., 1998; Woodward et al., 2010; Minneé et al., 2013; Lee et al., 2015b; Lee et al., In Press). Concentration of CP,

however, was generally at the lower end of the range reported in the literature, particularly for the non-legume species (Clark et al., 2010; Minneé et al., 2013; Bryant et al., 2014; Lee et al., 2015a; Minneé et al., 2017), which may reflect the soil N status at the experimental site or the cut and carry management regime the experiment was conducted under.

4.4.1 Crude protein and water-soluble carbohydrate release

The amount of CP released during maceration from the forages evaluated in this study varied 15-fold, with lucerne herbage releasing the most, up to 100 g of CP per kg DM more than from plantain which released the least. The ranking of the forages in terms of the amount of CP released (lucerne > ryegrass = clover > chicory > plantain) was similar to that observed by Burke (2004) although the values reported by Burke (2004) tended to be greater which may be due to differences in the methodologies employed between experiments (i.e. time of sampling, herbage maturity, different models of mincer for maceration, different laboratory methods for determining N content etc.). The extent of CP release from herbage cells is important for ruminant digestion as most forage N is readily fermented by rumen microflora, therefore the quantity released has potential to impact rumen ammonia concentration and in turn, urine N concentration (Huntington and Archibeque, 2000; Yang et al., 2010). The variation in CP release between forages appears to support studies showing reduced rumen ammonia and urine N concentrations (Soledad et al., 2016; Minneé et al., 2017), and differing patterns of diurnal urine N concentration (Bryant et al., under revision; Waghorn et al. under revision) from cows fed diets including plantain compared to those fed without, despite similar dietary N concentrations. The variation of CP released from herbage in this study may aid interpretation of differences in the nutritive value and N excretion between forages.

In contrast to CP, the release of WSC was extensive (> 85%) and similar among forages, compared with a range of 1 – 40% CP released. Previous studies have reported similarly extensive release, and thus used the release of soluble carbohydrates as a measure of the extent of cell rupture during comminution. Likewise, these other studies observed a similar disparity between WSC and CP release, which the authors attributed to the relative size of the molecules (Boudon and Peyraud, 2001; Boudon et al., 2006; Acosta et al., 2007). Water-soluble compounds (e.g. simple sugars) are

small (molar mass ~ 180 – 350 g/mol), thus they would require less extensive cell wall damage to be released compared with proteins, which are generally large (~ 60 000 – 100 000 g/mol) or associated with cell organelles (i.e. chloroplasts) (Goswami and Willcox, 1969). Non-protein N compounds, however, tend to be smaller than sugars (~ 17 – 65 g/mol), therefore it could be suggested that a large proportion of immediately released N are non-protein compounds. This may be relevant to studies of NUE, as NPN compounds are more rapidly fermented in the rumen compared to proteins. This theory is supported by the weakly positive, but significant, relationship observed between CP release and herbage NPN concentration (R^2 0.37; $P < 0.001$). This proposition and others in the literature agree that the type and quantity of compounds (i.e. WSC, CP) released during maceration depends on the magnitude of cell damage and rupture.

This study investigated forage effects on CP and WSC release, and demonstrated that release from lucerne, chicory and clover tended to be positively associated with herbage strength, but in ryegrass the relationship between release and strength was negative. Other research has demonstrated contrary associations, where nutrient release was weakly or negatively associated with tissue strength in grass species (Lees et al., 1981; Boudon and Peyraud, 2001; Acosta et al., 2007) but positively associated in red clover (*Trifolium pratense*) (Bryant, 1964). While no one characteristic was associated with nutrient release in all forages, those with lower CP and NPN concentrations tended to release the least CP, both proportionally and in terms of the total amount released (g/100 g DM). Within species, some strong relationships were evident. For example, CP concentration in herbage was strongly and positively correlated with amount of CP released from chicory, and release of WSC in ryegrass tended to be positively associated with WSC concentration and concentration of cell wall components (NDF, ADF and lignin). While no one herbage characteristic appeared to drive cell content release, the presence of significant relationships within some species may provide opportunities for manipulation of nutrient release through breeding or management.

The relative ratio of WSC: CP released by maceration, varied between forages. It has been suggested that the efficiency with which N and carbohydrates are utilised by ruminants is influenced by the release rate of these nutrients during digestion (Huntington and Archibeque, 2000; Phuong

et al., 2013). Tamminga et al. (1994) suggested a ratio of available carbohydrate to N of 5:1 was necessary for optimal microbial protein synthesis (MPS) and minimisation of N loss. The present study indicated that the quantity of CP released exceeded that of WSC in macerated lucerne (Figure 4.3), and this imbalance may explain the observed increase in N loss to urine in cows fed diets containing lucerne compared to those without (Waghorn et al., under revision). Conversely the ratio of quantities released from plantain was 17:1 which may explain the low rumen ammonia concentrations and reduced loss of N to urine in cows fed diets including plantain (Box et al., 2016; Bryant et al., 2017; Minneé et al., 2017). However, the WSC methodology does not measure all soluble carbohydrate compounds (e.g. pectins and ß-glucans), and plantain accumulates sorbitol (a sugar alcohol) which is not detected in the anthrone technique that was used to determine total WSC concentration in this study (Janeček et al., 2011). Therefore, it is likely that concentration and release of WSC is underestimated here. For this reason, ratios were recalculated to estimate total NFC, which altered the ratio but not the ranking of the forages, i.e. plantain and chicory tended to have ratios closer to the optima, while the legumes had the lowest ratios suggesting legumes are less N use efficient.

Contrary to expectations, the extent of comminution was not strongly associated with amount of CP release from herbage. For example, macerated plantain was fragmented to the greatest extent yet released the least CP. Assessment of the biomechanical properties of plantain in this study suggested that the herbage may be brittle (i.e. easily broken, cracked or snapped). Wright and Vincent (1996) suggested brittleness could result in fragmentation of the herbage occurring between cells, without rupturing the cells, meaning intercellular nutrients are not released. If this were the case, this would explain the low release of CP from plantain, but microscopic studies of macerated herbage are required for confirmation.

4.4.2 The biomechanical properties of herbage and the characteristics affecting them.

Clear differences in the biomechanical properties of herbage between the five forages evaluated were observed, as were differences between the different tissues within herbage (i.e. stem vs. leaf

tissue). The importance of herbage biomechanical properties lies in their resistance to breakdown during mastication and rumination, and effort required by the animal to harvest and digest herbage (MacKinnon et al., 1988; Easton, 1989; Wright and Illius, 1995). These factors will contribute to the palatability, intake rate and nutritive value of a forage (Moseley and Antuna Manendez, 1989; Mtengeti et al., 1996; Wilman et al., 1996). Previous studies have measured the strength of herbage to infer the relative resistance to fracture. In this study, leaf strength varied widely between species, with ryegrass leaf material markedly stronger than the other four evaluated. Similarly, lucerne stem was much stronger than stem of the other species. Within species, stem and petiole tissue required 3.5 – 10 times more force to punch than leaf material. This observation is consistent with other studies reporting psuedostem and stem tissue as 'stronger' than leaf tissue (Wright and Illius, 1995; Bryant et al., 2008), and is not unexpected as a key function of stem and petiole is to hold other plant tissues aloft for light capture or reproduction (i.e. wind or insect pollination). Relatively few comparisons of the strength of different tissues for other common species are available. While leaf tissue comprises the bulk of the ingested herbage of grazing livestock, they will also consume immature stem and petiole. The results present an opportunity for manipulating the strength of herbage through management strategies to alter the stem: leaf ratio in the sward, which could be used to manipulate the relative ease of comminution, and a means to influence CP and WSC release in some forages.

When the strength of herbage was calculated as a weighted average, the differences between the biomechanical properties of the five forages studied were less clear than when comparing leaf strength or herbage toughness using the mechanical macerator. There was a fivefold range in toughness observed (30 – 162 J/g FW) between the forages evaluated in this study. As the mechanical macerator used in this study macerates all the components of the sward simultaneously it may more accurately reflect the biomechanical properties of the herbage as experienced by a grazing animal that bites and masticates a mixture of plant tissues, rather than a calculated weighted average of strength which can be heavily skewed by large differences in punch force of some components in the sward. Previous studies have shown that the energy required to mill herbage was

negatively associated with the intake rate of sheep (Chenost, 1966) but positively associated to the amount of chewing an animal must do to form a bolus suitable for swallowing (Weston, 1985). These studies both used dried and ground herbage material for the assessment, but the mechanical macerator used in this study used fresh forage and may better reflect the herbage fed to livestock. The ability of the macerator used in this study to detect significant differences between the forages indicates that this technique could be a useful tool for screening herbage for differences in mechanical toughness in the first instance, before conducting studies using animals.

Investigation into the characteristics of the forages that contribute to herbage strength, showed that there were no common relationships between any single physical or chemical characteristic and strength between all five forages. When the relationships were explored within species, however, there were significant relationships between the weighted force to punch and herbage NDF and ADF concentration (fibre) in all forages except chicory. It must be noted, that the nature of the relationship varied between species: from a positive relationship between strength and fibre content in plantain, lucerne and clover, to a negative association in ryegrass. Similarly, relationships to explain variation in herbage toughness were conflicting. While the range in herbage toughness was large and highly correlated with the fibre concentration of herbage for all species, the relationship within species also varied from positive for lucerne and ryegrass, to non-existent in clover, chicory and plantain. Contrary relationships are also reported in the literature. Evans (1967a) and Inoué et al. (1994) described a positive relationship between fibre concentration and grass leaf strength, yet Bryant et. al. (2008) found no relationship between fibre concentration and force to break ryegrass. Therefore, the characteristics that influence herbage toughness and strength remain unresolved.

Lignin concentration was also not associated with herbage strength and toughness across forages. Lignin is a polymer present in cell walls to provide rigidity, yet this study showed that ryegrass herbage that contained the least lignin was the ‘toughest’, and conversely plantain that contained the greatest concentration of lignin was one of the weakest forages. These results show that while fibre and lignin concentrations explain some of the variation in biomechanical properties within some species, their concentration is of limited value for inferring herbage strength or toughness

when comparing forages. It is likely that the composition of the fibre and the configuration of the fibre-lignin matrix is important and further research into the anatomy of forage species is required to assist explanation of the variation in herbage biomechanics.

4.4.3 Comminution

Of the five species evaluated, the PSD of macerated plantain herbage was markedly different to the other herbages examined. Macerated plantain had a lesser proportion of large particles (those retained on a sieve with 2 mm apertures) and a greater proportion of small particles, whereas the opposite was true for ryegrass, chicory and clover which all had a similar PSD despite having quite different chemical and physical characteristics. When all species were evaluated, there were no relationships between any of the herbage characteristics measured and the extent of comminution. Thus, the reason for the differences in PSD were not clear. However, plantain herbage contained more lignin than the other herbages and while this was shown to be unrelated to herbage strength and toughness, it is possible that the increased lignin content or the nature of the lignin-fibre matrix in plantain could mean that the herbage is brittle (as described above) and readily fragmented in small pieces as shown by the PSD detailed in Table 4.5, and low herbage strength measured (Table 4.1).

Considering the wide range in the herbage biomechanical and chemical properties, the paucity of differences in PSD between species detected likely reflects the function of the apparatus used in this study. It appears that the mechanical macerator simply worked to an extent required (i.e. used more or less energy) to achieve a certain particle size within a certain range (defined by the sieve plates). The latter is appropriate for the original function for which the macerator was developed, which was to generate a PSD of herbage that was similar that of the rumen contents of sheep for feed digestibility studies. Therefore, while the use of this machine to detect differences in the herbage toughness and resistance to comminution may be of value, its use to imply differences in particle size between forages is limited.

Ryegrass was the only forage evaluated for which relationships between herbage chemical characteristics and the extent of comminution were observed. Ryegrass was also the only monocotyledon species evaluated in this study, (and thus has a different anatomical structure, i.e. parallel venation vs. branched in dicot species), as well as being considerably tougher and stronger compared with the other forages. These features may have made the herbage more resistant to comminution and allowed for differences in PSD of macerated herbage to be observed within ryegrass. The extent of comminution (the proportion of large particles) of ryegrass was strongly negatively associated with herbage strength. Yet, the extent of comminution was also positively associated with herbage toughness, NDF and lignin concentration. The two associations seem contradictory and suggest that in ryegrass, the two biomechanical properties measured (strength and toughness) are not complementary. However, it can be concluded from this study that ryegrass herbage with greater fibre and lignin concentration is tougher and more resistant to comminution which is likely to influence the grazing behaviour of livestock.

4.5 Conclusion

The aim of this study was to determine the variation in N (as CP) and WSC release between forages during maceration and investigate the chemical and physical characteristics that influence these processes. Better knowledge of the factors controlling variation in CP release between forages could assist our understanding of the FV of forages, and ability to manipulate N flow and nutrient use in livestock. Wide variation in the biomechanical properties, and release of CP and WSC between forages was observed, but relationships between characteristics were forage specific. Release of CP was not associated with the extent of forage comminution by maceration, nor were there any characteristics associated with release common to all forages. Attempts to use multiple regression analyses to predict cell content release from forage suggest relationships are species specific, and cannot be applied to other species with confidence. Within forages, however, associations between release and herbage strength, fibre and lignin concentrations and amount of CP and WSC released were observed. This provides avenues for manipulation of certain forages by altering management practices or breeding to alter herbage chemistry and thus nutrient release. For example, it was

discussed in the literature review (section 2.7) that increasing N fertiliser applied to grass swards increases herbage N and soluble N concentration, as well as improving herbage degrability. Therefore, based on the results of this study it could be suggested that increasing N fertiliser applied to other forage species swards would also improve herbage degrability and the release of nutrients, but requires investigation.

5

The effect of nitrogen fertiliser rate on herbage characteristics, nitrogen release and degradation of four forages.

5.1 Introduction

The productivity of pasture-based livestock systems is closely related to the amount, and quality of herbage grown on farm (Thom, 2000; Wales and Kolver, 2017). In the 1990s, nitrogen (N) fertiliser became widely available in New Zealand and was rapidly adopted by farmers as a simple and cost-effective tool to increase herbage growth and in turn, increase livestock production (per ha) (Clark et al., 2007; WRC, 2015). Between 1990 and 2012, cow numbers doubled (DairyNZ, 2014a), in part facilitated by the additional feed grown on farm. Over a similar period, the amount of N leached from agriculture per annum increased by 29%, to a total of 1.5 million kg N/y (MFE, 2015). This leaching loss is a significant environmental concern and highlights the inefficiency of N utilisation in these systems.

Direct leaching of fertiliser N can occur at high application rates (~ 10% at 400 kg N/ha/y), but the main source of leached N is from urine excreted from livestock (Ledgard et al., 1999a). This is because N loading from urine can be very high (up to 1000 kg N/ha, Di and Cameron (2007)) beyond which forage plants are unable to fully utilise, particularly during periods of high rainfall (leading to greater drainage of water from the soil) and cooler soil temperatures. The high N loading from urine occurs because ruminant livestock poorly utilise N, particularly in intensively managed grazing systems (13 – 28% utilisation (see Chapter 2, section 2.5)) where herbage CP concentration is typically high (> 20%), and thus dietary N intake, is often in excess of livestock requirements (Kolver and Muller, 1998). Therefore, a balance must be found between using N fertiliser to optimise growth but minimise excess dietary N.

Generally, increasing fertiliser N applied to swards increases DM yield, herbage N concentration and the solubility of herbage N in grass-based swards (Goswami and Willcox, 1969; Wilman and Wright, 1986; Bryant et al., 2012). Because forage grasses readily accumulate N from the soil, herbage N concentration continues to increase beyond the point when gains in DM yield cease (Whitehead, 2000). Furthermore, accumulated N that exceeds plant growth demand, is largely stored as nitrate in the plant cells, which is highly soluble (Whitehead, 2000) and rapidly degraded in the rumen following ingestion (Ulyatt, 1997). When total herbage N exceeds animal requirements, N utilisation declines and N excreted in urine increases (Jarvis et al., 1989; Haynes and Williams, 1993), ultimately leading to exponential increases in N leaching (Figure 5.1) (Kebreab et al., 2001; Pacheco and Waghorn, 2008). Thus, defining the effects of increased rate of N fertiliser application on herbage chemical composition, CP solubility and degradability could enable improved N fertiliser management to reduce N excretion whilst maintaining animal production.

To date, much of the research investigating the effect of N fertiliser application rate on forages has been concerned with grass species, predominantly, perennial ryegrass (Hoekstra et al., 2008; Bryant et al., 2012). Because there is an increased use of non-ryegrass species in New Zealand there is a need to widen our understanding of the effect of N fertiliser on herbage growth and chemical composition of other common species. Forage species naturally differ in their N concentration, and in their capacity for accumulating N and nitrate (Murphy and Smith, 1967), thus optimal N fertiliser rate may differ between species and furthermore, the responses (chemical and physical) of those species may differ to that for ryegrass.

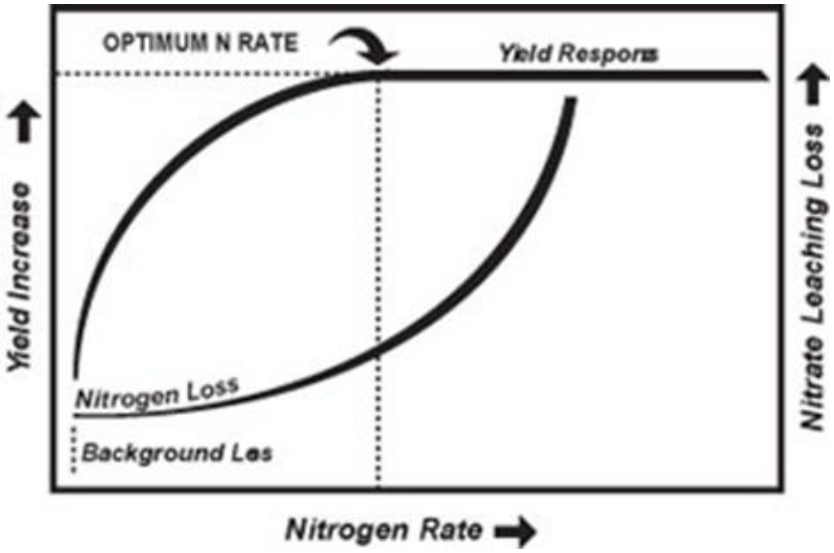


Figure 5.1 Generalised relationship between the amount of fertiliser N applied (kg N/ha) and herbage yield and nitrate leaching (Adapted from: DEFRA, 2011, © Crown copyright 2010).

The study described in Chapter 4 indicated that the physical release of soluble CP by cell rupture was influenced by chemical and physical characteristics of the herbage, which differed between forages. For example, crude protein and non-protein nitrogen concentration was positively correlated with CP release in some forages, whereas dry matter content and herbage biomechanical properties explained at least 30% of the variation in CP release in others. This could provide an opportunity to alter the release of CP and carbohydrate from herbage to reduce urinary N excretion by manipulating forage characteristics through fertiliser N management. Thus, the aim of the experiment detailed in this chapter was to quantify the effect of N fertiliser application rate (0 – 350 kg N/ha/y) on herbage chemical and physical characteristics, CP release and degradation rate of CP from macerated chicory, lucerne, plantain and ryegrass herbage. This knowledge could help inform fertiliser management strategies to provide positive outcomes for farm productivity and environmental impact.

5.2 Materials and Methods

5.2.1 Experimental design and harvest dates

Details of forages used, sward establishment, harvest criteria and sampling are described in Chapter 3. The four forages used in this study were: chicory, lucerne, plantain and perennial ryegrass (ryegrass). The four N fertiliser rates were 0, 100, 200 and 350 kg N/ha/y (hereafter referred to as 0N, 100N, 200N and 350N). Effects of treatments (except the *in sacco* degradation study) were measured in autumn (May) and spring (November) of 2015 when uptake of N from soil by plants is anticipated to be low and high, respectively, as affected by soil temperature and herbage growth rate. Fertiliser N applications were evenly distributed over the year and occurred after each harvest (Table 5.1). At the time of autumn sampling, the 0N, 100N, 200N and 350N treatments plots had received 0, 50, 100 and 175 kg N/ha respectively; and in spring a total of 0, 90, 180 and 315 kg N/ha had been applied to the respective treatments, since establishment in October 2014.

Table 5.1. Amount of nitrogen applied after each harvest for each species and treatment.

		N application (kg N/ha) after each harvest			
Species	No. harvests/y	0N	100N	200N	350N
Chicory	8	0	12.5	25	44
Lucerne	8	0	12.5	25	44
Plantain	10	0	10	20	35
Perennial ryegrass	10	0	10	20	35

Accumulated growing degree days (GDD, >5°C for all species as per Powell et al. (2007)) following the previous harvest were, 478 GDD for chicory and lucerne, and 457 GDD for plantain and ryegrass in autumn; and in spring were 298, 356, 194 and 262 GDD for chicory, lucerne, plantain and ryegrass, respectively.

5.2.2 Herbage physical and chemical characterisation

The methodologies used to determine herbage mass, and physical and chemical characteristics are detailed in section 3.2.4 (herbage dry matter (DM) yield, DM content and botanical composition), section 3.3 (herbage biomechanical properties), section 4.2.2 (morphology) and section 4.2.3 (herbage chemical composition). Because concerns were raised in Chapter 4 that the WSC assay does not measure all soluble carbohydrates, herbage non-structural carbohydrate (NSC) concentration which represents readily fermentable carbohydrates, was determined by Equation 4.1 (section 4.3.1).

5.2.3 Herbage comminution and CP and WSC release

The methodologies used for evaluating the extent of comminution of the herbage and release of CP and WSC by maceration are detailed in Chapter 4. In brief, the extent of mechanical comminution was determined by wet sieving macerated herbage to separate material into five particle size fractions and a sixth 'soluble' fraction, that was calculated by the difference between the amount of DM sieved and the sum of the DM retained on the five sieves. Material retained on each sieve was dried to determine particle size distribution (PSD) on a dry weight basis.

Release of CP and WSC was determined by analysing the concentration of CP and WSC in both intact herbage and macerated material that had been rinsed. The proportion of CP and WSC released was calculated by difference (methodology detailed in section 4.2.4).

5.2.4 In sacco degradation of herbage

Kinetics of DM and N degradation of the macerated herbage were measured in four fistulated cattle by *in sacco* incubation over 72 h as per Burke et al. (2002) and Chaves et al. (2006). The incubation was undertaken in September 2016 with lactating, Holstein × Friesian crossbred cows (536 ± 38 kg live-weight) with permanent rumen fistula, from the DairyNZ experimental herd. The cows grazed a common allowance (20 kg DM/cow/d) of perennial ryegrass/white clover herbage during the period of incubation. Macerated material from the autumn sampling (2015) was used, and sufficient fresh material equating to approximately 3 g of DM was weighed into 100 x 100 mm Dacron bags

(mean pore size 35 µm; Ankom Technology, USA). Each bag was heat-sealed and stored at -20°C until the day of incubation.

Approximately 15 minutes before incubation, bags were thawed and then seven bags from each treatment were placed into 20 × 30 cm mesh bags containing ~ 300 g of stainless steel weights, which allowed for easy retrieval of the nylon bags from the rumen and held the bags in the centre of the rumen of the cow. One bag of each treatment was removed from the rumen of each cows after 2, 4, 6, 9, 12, 24 and 72 h. One additional bag of each treatment that was not placed in the rumen (0 h; pre-degradation sample), and the rumen-incubated bags, were hand-rinsed in cold water until no further colour appeared. Bags were dried at 60°C for 48 h then weighed to determine DM loss, then residues were ground to pass a 1 mm sieve (Thomas Wiley Mill, USA) for estimation of N content by near infrared spectroscopy (NIRS; Massey University, Palmerston North, New Zealand). NIRS spectra were collected on all samples using a multi-purpose analyser 0172.04 with Spectroscopy Software OPUS 7.0 (Bruker Optic GMBH, Madison, Wisconsin, USA). NIRS calibrations for crude protein (CP) concentration are based on N concentration data determined using a Carlo Erba NA1500 elemental N analyser, × 6.25 (AOAC 1990) from a large database of samples (Corson et al., 1999). Standard errors of prediction for CP were 1.13% of DM, with resultant correlation (R^2) of 0.97.

5.2.5 Statistical analyses

Data were analysed for variance (ANOVA) using a mixed models approach in SAS version 9.3 (SAS Institute Inc., NC, USA) with forage species and N fertiliser rate and their interaction as fixed effects and block as a random effect. ANOVA was followed by Tukey's t-test for pairwise comparisons where $P < 0.05$ is considered significant.

Data obtained from the *in sacco* incubations were plotted against time and a mathematical model (Dhanoa et al., 1995) fitted to the resulting curve for each forage to describe the digestion kinetics of DM and CP. The model is summarised as:

$$y = A + B\left(1 - e^{-kt - d\sqrt{t}}\right)$$

Equation 5.1

Where *A* = soluble fraction (% of DM or CP at t = 0), *B* = degradable insoluble fraction, *t* = time (h) and *d* is a constant. Parameter estimates of k (%/h) for DM and CP were derived.

5.3 Results

5.3.1 Herbage mass and characteristics

5.3.1.1 DM content and herbage mass

A significant interaction between N fertiliser application rate and forage species for herbage mass (kg DM/ha) was observed in both seasons. Herbage mass of chicory, plantain and ryegrass increased with increasing N applied, but herbage mass of lucerne was unaffected (Figure 5.2). While mass increased with increasing N between 0 and 200N in all non-legume forages, the effect of increasing fertiliser N rate beyond 200N was inconsistent between forages. Plantain continued to accumulate mass beyond 200N in both seasons, while increasing N fertiliser beyond 200N improved chicory yield in spring but not autumn, and did not increase ryegrass yield in either season. The response to increasing N fertiliser application is presented in Table 5.2, and shows that the relative response of DM per unit of N applied declined in chicory, plantain and ryegrass at fertiliser N rates exceeding 200N in both seasons.

Increasing the rate of N application progressively reduced the DM content of ryegrass, chicory and plantain in both autumn and spring ($P < 0.001$; $R^2 > 0.85$). Dry matter content of lucerne herbage was unaffected by N fertiliser application rate (Figure 5.2).

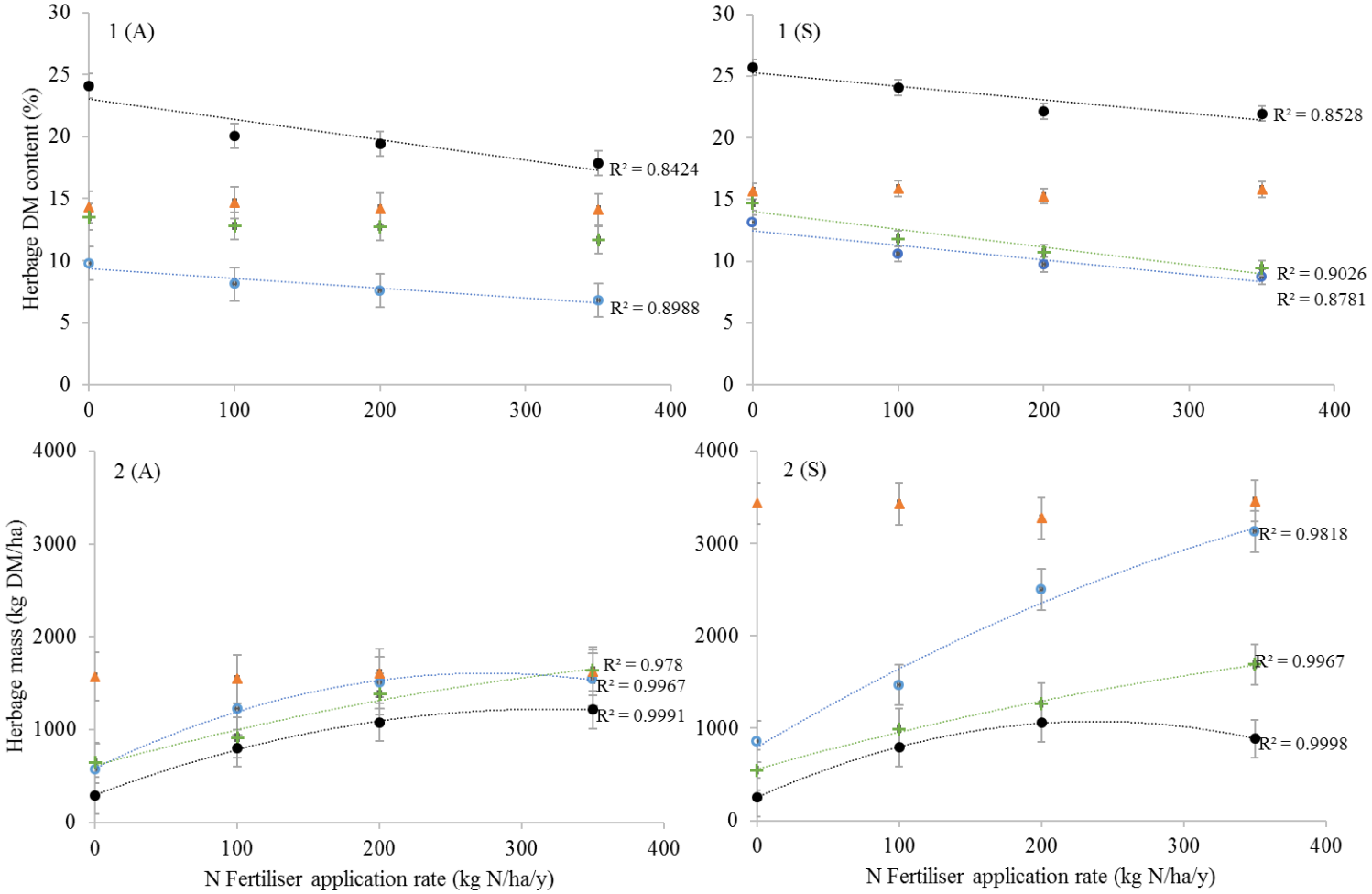


Figure 5.2 Herbage DM content (1), and mass (2) determined in autumn (A), and spring (S) for swards of chicory (○), lucerne (▲), perennial ryegrass (●), and plantain (+). Bars indicate standard error and R^2 values are given when the relationship is significant ($P < 0.05$). Trend lines are presented when the main effect of N fertiliser rate was statistically significant.

Table 5.2 Additional dry matter (DM) yield gained with increasing fertiliser N rates compared to the 0N treatment (kg DM/ha) and response to N fertiliser applied (g DM/kg N).

	N applied at each harvest (kg N/ha)				DM yield (kg DM/ha)				Additional DM yield above 0N (kg DM/ha)			Response (kg DM/kg N)		
N fertiliser application rate (kg N/ha/y)	0	100	200	350	0	100	200	350	100	200	350	100	200	350
Autumn														
Chicory	0	12.5	25	44	573	1225	1504	1539	652	931	967	52	37	22
Lucerne	0	12.5	25	44	1570	1545	1607	1627	-25	37	57	-2	1	1
Plantain	0	10	20	35	641	914	1385	1637	273	744	996	27	37	28
Perennial ryegrass	0	10	20	35	290	801	1079	1214	511	790	924	51	39	26
Spring														
Chicory	0	12.5	25	44	859	1469	2501	3132	610	1642	2273	49	66	52
Lucerne	0	12.5	25	44	3434	3428	3272	3461	-6	-162	27	0	-6	1
Plantain	0	10	20	35	547	993	1265	1689	446	718	1142	45	36	33
Perennial ryegrass	0	10	20	35	253	792	1059	888	539	806	635	54	40	18

5.3.1.2 Botanical composition and morphology

Increasing N fertiliser rate increased the proportion of green leaf in swards of plantain and ryegrass in spring (from 0.65 – 0.77 and 0.71 – 0.85 respectively ($P < 0.05$)), and was accompanied by small non-significant declines in the proportion of dead and stem material in the herbage (Appendix B.3). The composition of lucerne and chicory herbage were unaffected by N fertiliser rate.

Relationships between N fertiliser and leaf size were similar in spring and autumn (Figure 5.3). There was an interaction ($P < 0.001$) between N fertiliser rate and forage for leaf width in both seasons, where leaf width of the herb species, chicory ($P < 0.001$), and plantain ($P = 0.001$), increased with increasing N rate. Whereas, leaf width was unaffected by N rate in ryegrass and lucerne. Leaf length progressively increased with increasing N rate in all non-legume species, in both seasons (Figure 5.3).

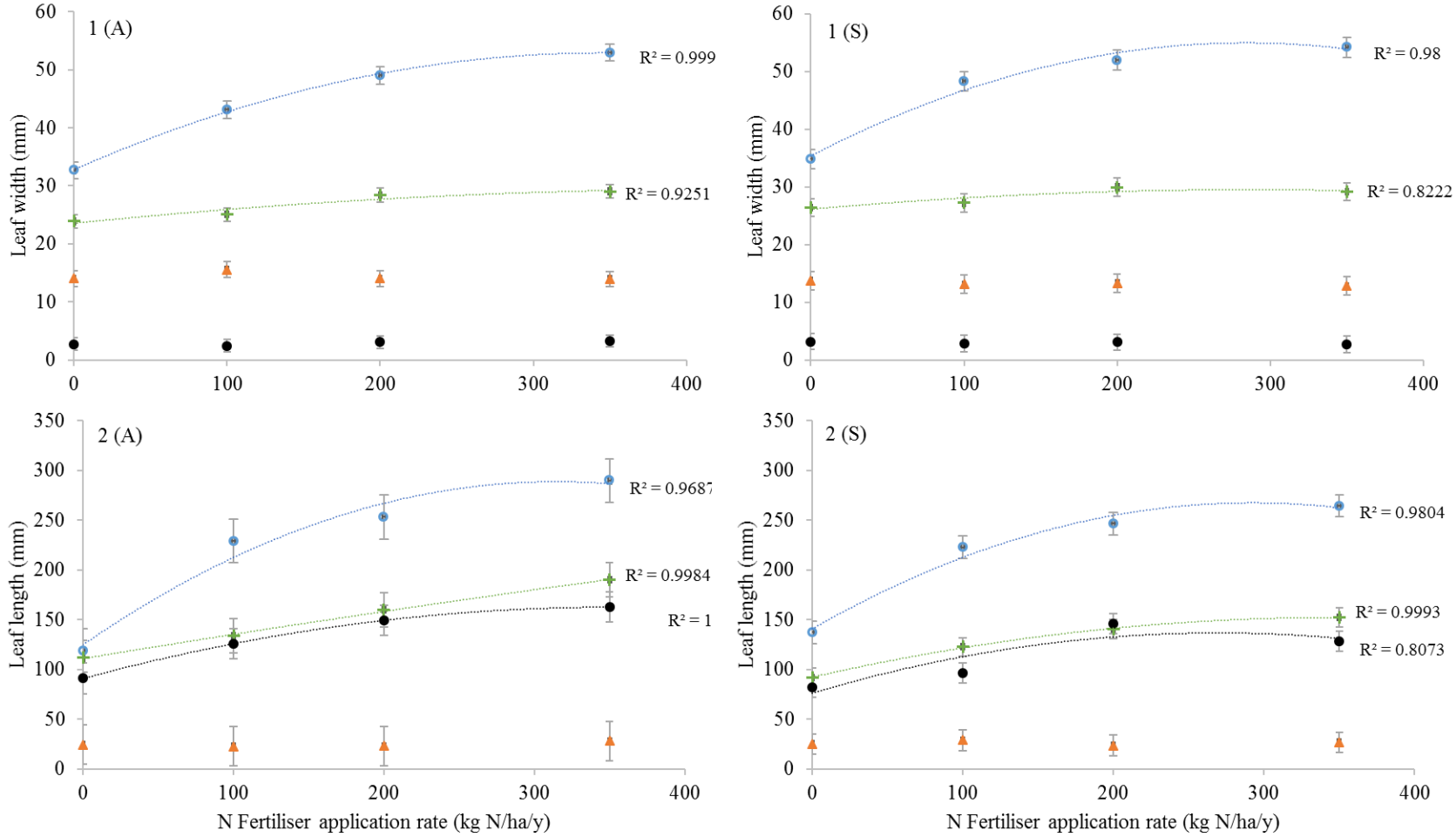


Figure 5.3 Leaf width (1) and length (2) of herbage collected in autumn (A) and spring (S) from swards of chicory (○), lucerne (▲), perennial ryegrass (●), and plantain (+). Bars indicate standard error, and trend lines and R^2 values are given when the relationship is significant ($P < 0.05$).

5.3.1.3 Herbage biomechanical properties

A forage × N fertiliser rate interaction for strength ($P < 0.001$) showed that ryegrass was the only species where herbage strength was affected by N fertiliser rate. Increasing N fertiliser reduced the strength (force to punch) of both leaf material and herbage of ryegrass ($P < 0.001$ Figure 5.4), though the relationship was more evident in spring compared with autumn. Linear regression analyses revealed that the strength of ryegrass leaf and herbage was positively correlated with herbage DM content and NDF concentration (Appendix Table B4; $P < 0.001$).

Herbage toughness (maceration energy, J/g FW) declined with increasing N fertiliser rate in ryegrass herbage in both seasons, and chicory and plantain herbage in spring (Figure 5.4). As with strength, the decline in ryegrass toughness with increasing N fertiliser rate was greater in spring than in autumn, and was positively correlated with herbage DM content. The biomechanical properties of lucerne were not affected by N fertiliser rate.

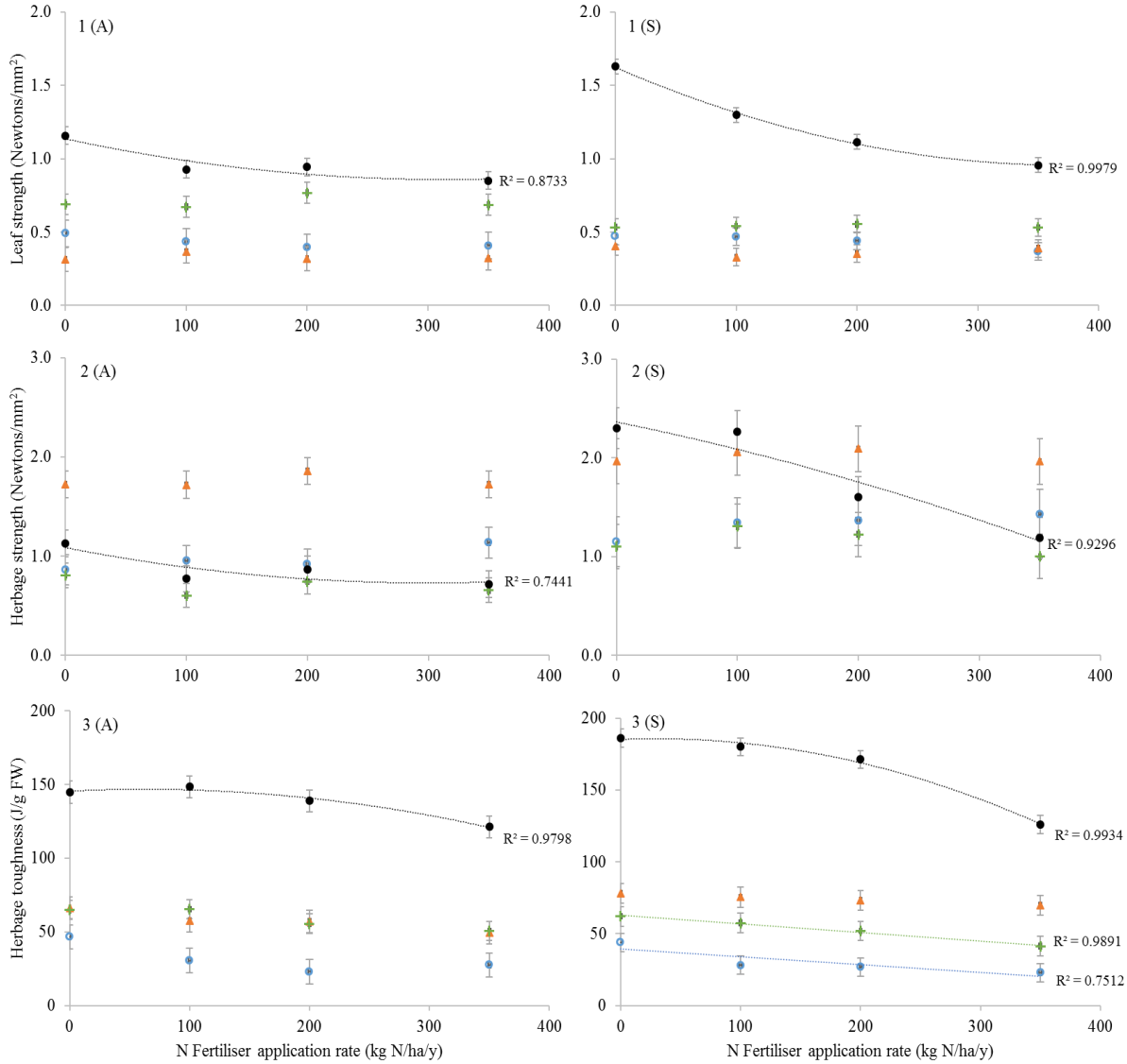


Figure 5.4 Strength of leaf (1) and herbage (2), and herbage toughness (3) of herbage collected in autumn (A) and spring (S) from swards of chicory (○), lucerne (▲), perennial ryegrass (●), and plantain (+). Bars indicate standard error, and trend lines R^2 values are given when the relationship is significant ($P < 0.05$).

5.3.1.4 Nitrogen and carbohydrate fractions

Herbage nitrogen and carbohydrate composition data are presented in Tables 5.2 and 5.3, respectively. There was an interaction between N fertiliser rate and forage on total CP concentration in herbage in autumn (P = 0.007) but not in spring. In autumn, increasing fertiliser N application rate from 0 – 350N increased in herbage CP content in ryegrass and plantain but not in chicory or lucerne. In spring, however, increasing fertiliser N application tended to increase herbage CP concentration in all forages, but was only significant for ryegrass and chicory.

The effect of increasing N fertiliser rate on the N associated with fibre (NDIN) varied between forages in spring (N × forage, P = 0.019); NDIN tended to increase with increasing N rate in chicory and ryegrass, but decrease in lucerne with no trend in plantain. There was no effect of N fertiliser on NDIN concentration on any forage in autumn.

Herbage nitrate concentration increased with increasing N fertiliser rate in all forages in both seasons (N × forage P > 0.05) by between 66 – 167% (range from 0 – 0.71 g/100g DM). The effect of increasing N fertiliser rate on nitrate concentration, however, only achieved significant levels (P < 0.05) in autumn ryegrass, and spring lucerne herbage.

There were few effects of N fertiliser rate on carbohydrate fractions. There were no N fertiliser rate × forage interaction for fibre concentration (NDF), however there was a trend (P < 0.10) for decreasing NDF concentration in ryegrass herbage with increasing fertiliser N rate in spring (decreasing from 42 – 36 g/100 g DM). Water soluble carbohydrate concentration increased by 59% in autumn for chicory herbage in the 350N treatment compared with unfertilised chicory (0N), but WSC concentration was unaffected by N rate in the other forages.

Table 5.3 Concentrations of crude protein and nitrogen fractions (g/100 g DM) in chicory, lucerne, plantain and perennial ryegrass herbage harvested in autumn and spring from swards fertilised at four rates of nitrogen fertiliser.

	N rate (kg N/ha/y)							*p* - Value		
	0	100	200	350	LSM	SED	*p* - Value	Forage	N rate	Forage x N
Autumn										
Crude protein								< 0.001	0.005	0.007
Chicory	15.9^{b}	15.5^{b}	18.1^{a}	18.3^{a}	17.5^{B}	1.09	0.039			
Lucerne	26.8	28.6	27.1	27.9	27.6^{A}	0.97	0.346			
Plantain	12.0^{b}	14.5^{ab}	14.6^{ab}	16.3^{a}	14.4^{C}	0.87	0.005			
Perennial ryegrass	17.5^{b}	17.5^{b}	17.4^{b}	21.8^{a}	18.6^{B}	1.21	0.001			
Neutral detergent insoluble N								0.057	0.911	0.371
Chicory	0.66	0.45	0.36	0.50	0.49^{AB}	0.27	0.715			
Lucerne	0.22	0.33	0.21	0.27	0.26^{B}	0.24	0.964			
Plantain	0.62	0.99	0.95	0.54	0.78^{A}	0.22	0.070			
Perennial ryegrass	0.77	0.54	0.50	0.81	0.65^{A}	0.30	0.648			
Nitrate								0.038	0.003	0.317
Chicory	0.14	0.11	0.22	0.28	0.19^{B}	0.108	0.447			
Lucerne	0.28	0.37	0.51	0.48	0.41^{A}	0.096	0.104			
Plantain	0.09	0.10	0.10	0.15	0.11^{B}	0.086	0.840			
Perennial ryegrass	0.00^{b}	0.11^{ab}	0.24^{ab}	0.38^{a}	0.18^{B}	0.117	0.009			
Spring										
Crude protein								< 0.001	< 0.001	0.531
Chicory	13.2^{ab}	12.3^{b}	17.4^{a}	16.2^{ab}	14.7^{B}	2.096	0.033			
Lucerne	24.4	25.0	26.0	26.6	25.5^{A}	1.912	0.645			
Plantain	14.9	14.7	16.4	19.1	16.3^{B}	1.864	0.064			
Perennial ryegrass	11.9^{b}	12.3^{b}	13.8^{ab}	17.7^{a}	13.9^{B}	1.719	0.008			
Neutral detergent insoluble N								< 0.001	< 0.001	0.019
Chicory	0.10^{b}	0.17^{b}	0.26^{b}	0.65^{a}	0.29^{B}	0.115	0.007			
Lucerne	0.47^{a}	0.17^{b}	0.17^{b}	0.20^{b}	0.25^{B}	0.102	0.048			
Plantain	0.65^{a}	0.47^{ab}	0.26^{b}	0.58^{a}	0.49^{A}	0.098	0.004			
Perennial ryegrass	0.45^{b}	0.43^{b}	0.52^{ab}	0.69^{a}	0.52^{A}	0.085	0.021			
Nitrate								< 0.001	0.002	0.332
Chicory	0.12	0.14	0.12	0.13	0.13^{B}	0.114	0.998			
Lucerne	0.27^{b}	0.51^{ab}	0.66^{a}	0.72^{a}	0.54^{A}	0.101	0.001			
Plantain	0.00	0.08	0.12	0.25	0.10^{B}	0.097	0.075			
Perennial ryegrass	0.04	0.04	0.07	0.11	0.07^{B}	0.084	0.819			

LSM, Least squares mean; SED, Standard error of the difference. Note: Means in rows sharing the same lower case superscript are not significantly different, means in columns sharing the same upper case superscript are not significantly different.

Table 5.4 Concentration of neutral detergent fibre and water-soluble carbohydrate (g/100 g DM) in chicory, lucerne, plantain and perennial ryegrass herbage harvested in autumn and spring from swards fertilised at four rates of nitrogen fertiliser.

	N rate (kg N/ha/y)							*p* - Value		
	0	100	200	350	LSM	SED	*p* - Value	Forage	N rate	Forage x N
Autumn										
Neutral detergent fibre								< 0.001	0.060	0.150
Chicory	19.7	22.3	18.7	23.9	21.1^{B}	2.03	0.078			
Lucerne	28.7	26.7	26.2	27.3	27.2^{B}	1.80	0.572			
Plantain	24.7^{ab}	27.0^{a}	22.4^{b}	24.9^{ab}	24.8^{B}	1.61	0.060			
Perennial ryegrass	46.5	41.9	44.0	44.1	44.1^{A}	2.24	0.451			
Water-soluble carbohydrate								< 0.001	0.269	0.080
Chicory	8.7^{b}	11.2^{ab}	11.6^{ab}	13.9^{a}	11.3^{B}	1.26	0.032			
Lucerne	9.4	9.7	9.1	11.1	9.8^{B}	1.12	0.303			
Plantain	15.6	15.3	15.0	14.3	15.1^{A}	1.01	0.519			
Perennial ryegrass	14.5	16.1	15.2	13.3	14.8^{A}	1.40	0.246			
Non-structural carbohydrate								< 0.001	0.035	0.414
Chicory	38.8	38.0	39.9	35.8	38.1^{AB}	2.60	0.532			
Lucerne	31.4	31.8	33.8	31.9	32.2^{B}	2.27	0.733			
Plantain	41.5	38.5	43.1	39.2	40.6^{A}	2.02	0.120			
Perennial ryegrass	24.4^{a}	23.7^{ab}	22.4^{ab}	17.9^{b}	22.1^{C}	2.52	0.045			
Spring										
Neutral detergent fibre								< 0.001	0.265	0.090
Chicory	22.1	23.1	24.7	22.0	23.0^{C}	2.44	0.610			
Lucerne	29.0^{ab}	30.7^{a}	24.6^{b}	28.0^{ab}	28.1^{B}	2.22	0.057			
Plantain	21.9	25.1	26.1	23.3	24.1^{C}	2.17	0.288			
Perennial ryegrass	42.4^{a}	40.6^{ab}	39.9^{ab}	36.0^{b}	39.7^{A}	1.99	0.023			
Water-soluble carbohydrate								< 0.001	0.697	0.502
Chicory	12.0	16.1	12.6	14.8	13.9^{B}	1.89	0.072			
Lucerne	9.5	8.6	11.0	9.1	9.6^{C}	1.72	0.542			
Plantain	15.0	14.8	13.8	14.2	14.4^{B}	1.68	0.909			
Perennial ryegrass	23.0	23.9	23.0	21.3	22.8^{A}	1.55	0.431			
Non-structural carbohydrate								0.002	0.424	0.559
Chicory	45.3	44.3	39.1	42.9	42.9^{A}	4.62	0.372			
Lucerne	32.9	30.8	36.0	32.4	33.0^{B}	4.10	0.614			
Plantain	45.9	42.9	38.4	38.6	41.4^{A}	3.94	0.269			
Perennial ryegrass	31.9	34.0	32.2	31.6	32.4^{B}	3.40	0.911			

LSM, Least squares mean; SED, Standard error of the difference. Note: Means in rows sharing the same lower case superscript are not significantly different, means in columns sharing the same upper case superscript are not significantly different.

5.3.1.5 Carbohydrate-to-protein ratio

The WSC: CP ratio among the four forages and four N fertiliser application rates ranged from 0.34 – 1.95. Changes in the CP concentration of chicory, lucerne and ryegrass herbage with fertiliser N application rate resulted in changes in the WSC: CP ratio (Figure 5.5). In general, the WSC: CP ratio declined with increasing N application in plantain and ryegrass herbage but was variable for chicory. For example, increasing the fertiliser N applied to ryegrass herbage from 0 to 350N resulted in a 38% decline in the ratio. In lucerne herbage the WSC: CP was similar between N rates (mean 0.36). In recognising that WSC may underestimate fermentable carbohydrate in some forages, NSC: CP was calculated, and showed the same trend as the WSC: CP of declining ratio with increasing N rate in plantain and ryegrass herbage. However, between forages, the ratio was markedly improved when NSC was used in place of WSC for chicory and plantain relative to ryegrass and lucerne.

5.3.2 Crude protein and WSC release with mechanical maceration

There were no forage × N rate interactions observed for the proportional WSC release, which was unaffected by N fertiliser rate. The proportional release of CP in response to increasing fertiliser N, however, differed between forages in both seasons (species × N rate $P < 0.05$). Increasing N fertiliser rate increased the proportion of CP released from ryegrass herbage in both seasons, by 74 and 61% in autumn and spring, respectively. While, proportional release of CP from plantain increased with increasing fertiliser N application rate in autumn (0.02 – 0.15; SED = 0.04, $P = 0.0094$) but not in spring; and the proportional release of CP from chicory and lucerne was unaffected in either season. For the forages where proportional CP release was affected by N fertiliser application, CP release was positively correlated ($P < 0.05$) with herbage CP and nitrate concentration.

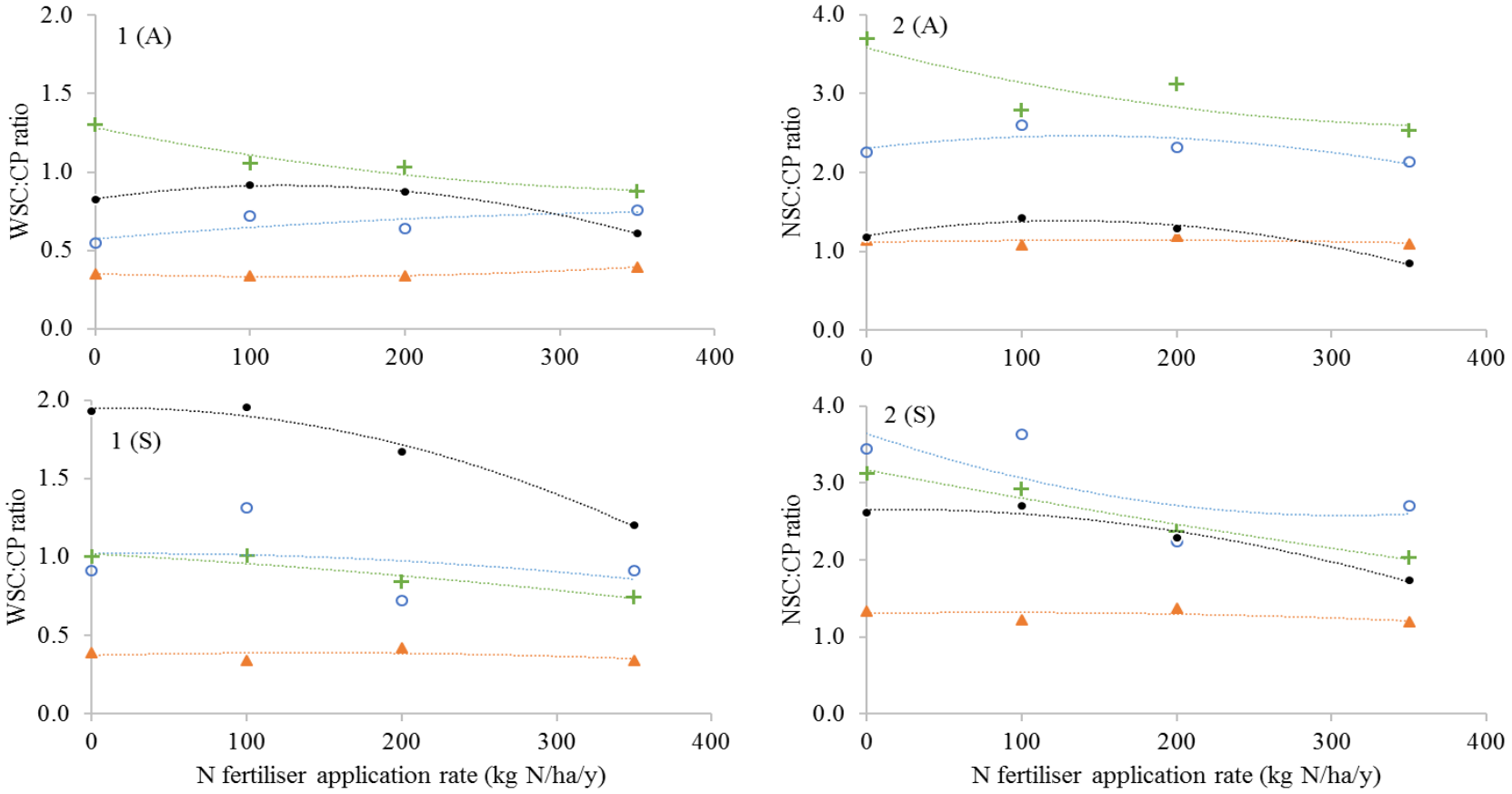


Figure 5.5 Ratio of water soluble carbohydrate (WSC): crude protein (CP) (1) and Non-structural carbohydrate[1] (NSC): CP (2) plotted in relation to the N fertiliser application rate from herbage collected in autumn (A) and spring (S) from chicory (○), lucerne (▲), plantain (+) and perennial ryegrass (●). Bars indicate standard error, and trend lines R^2 values are given when the relationship is significant ($P < 0.05$).

[1] NSC is calculated as NSC = 100 – (CP + NDF + Ash + Lipid).

The amount (g/ 100 g DM) of CP released is illustrated in Figure 5.6 and followed a similar trend to observed proportional loss of CP. The amount of CP released from ryegrass in response to increasing N fertiliser rate differs to that from the other forages in this study ($P < 0.05$), where CP release increased by 3.5 and 4.1 g CP per 100 g of DM with increasing fertiliser N rate (0 – 350N) in autumn and spring, equating to increases of 141 and 74% respectively. The amount of CP released from plantain increased six-fold, from 0.49 – 3.32 g CP/100 g DM when fertiliser increased from 0 – 350N ($P = 0.04$) in autumn, but was unaffected by N fertiliser in spring. The release of CP from chicory was variable, peaking at 6.5 g CP/100 g DM at 200N in autumn, with a trend ($P < 0.10$) toward increasing amount CP released at fertiliser rates exceeding 100N in spring. Release of CP from lucerne herbage was not affected by N fertiliser rate in either season.

5.3.3 Particle size distribution of macerated herbage

There was an effect of forage on PSD with trends similar to those reported previously (sections 4.3.3 and 4.4.3). Macerated plantain contained a lesser proportion (by DM) of large particles (> 2 mm) than the other forages (14 vs 23% of DM; Appendix Table B.5). There was no interaction between N fertiliser and species for PSD and generally there was little impact of N fertiliser on PSD for chicory, plantain and lucerne. The one exception was in spring where ryegrass herbage in the 0N fertiliser treatment had a greater proportion of large particles (> 2mm) in macerated material compared with fertilised ryegrass herbage (0.27 vs mean 0.18).

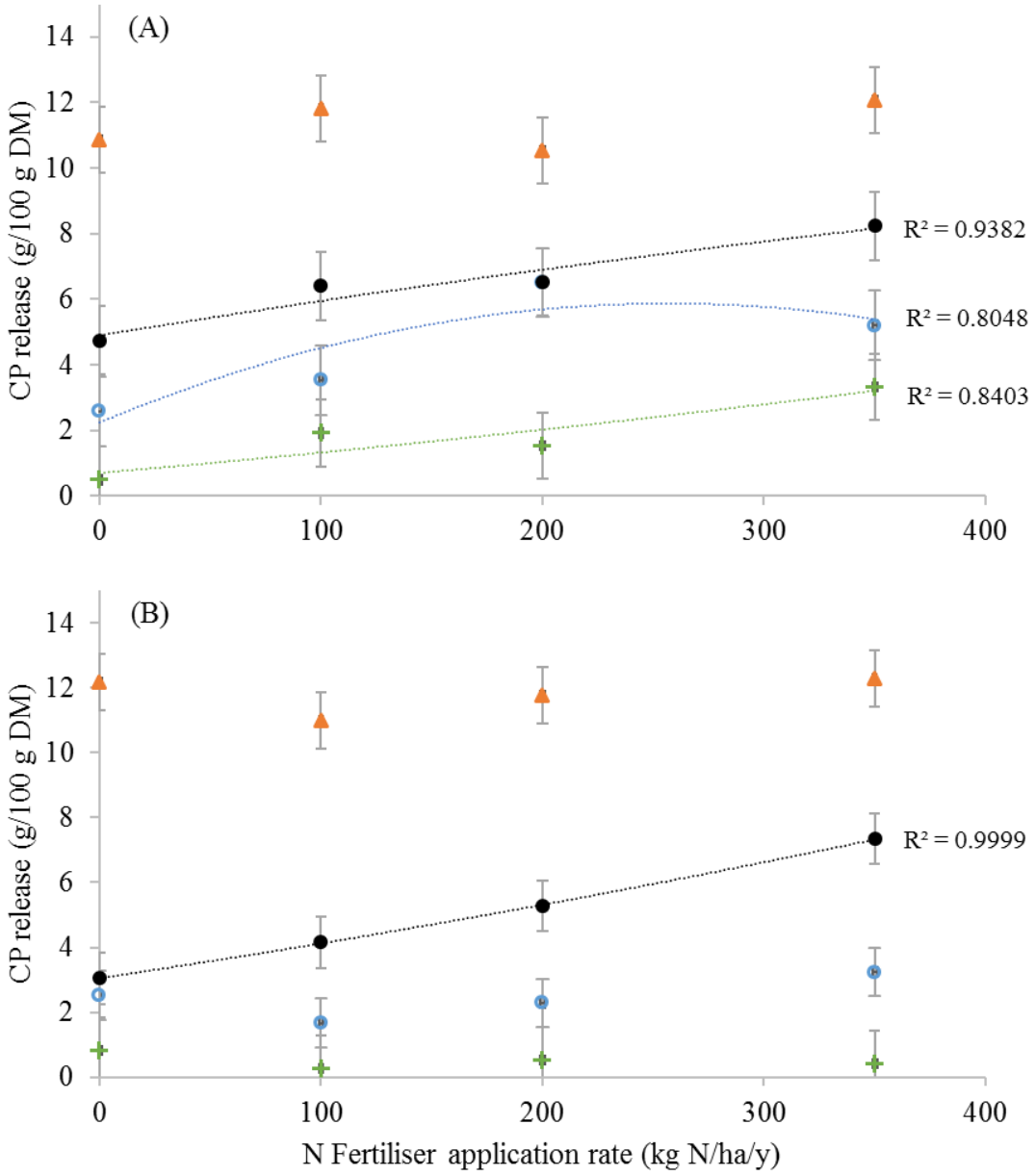


Figure 5.6 Crude protein (CP) release (g/100 g DM) during maceration of herbage collected in autumn (A) and spring (B) from swards of chicory (○), lucerne (▲), plantain (+) and perennial ryegrass (●). Bars indicate standard error, and trend lines R^2 values are given when the relationship is significant ($P < 0.05$).

5.3.4 DM and CP degradation kinetics

The effect of N fertiliser rate on herbage DM solubility ('A' fraction) differed between forage (P forage × N rate < 0.001). The DM in the A fraction ranged from 28% (lucerne) to 37% (chicory) of the DM across forages (Table 5.5). Soluble DM increased by approximately 11% when N fertiliser

rate increased from 0 – 350N in chicory and ryegrass herbage. In contrast, DM solubility was reduced by approximately 5% in plantain at the greater rates of N fertiliser compared with lesser rates. Lucerne DM solubility was unaffected (Table 5.5). There were no effects of N fertiliser rate on the proportion of degradable (B) DM, undegradable fraction (U), or the rate of DM disappearance (k%/hr, Table 5.5), and no forage × N rate interactions were observed for these parameters ($P > 0.05$).

However, the effect of N fertiliser on all CP degradation parameters differed between forage (P forage × N rate < 0.05). Only ~ 14% of CP was in the soluble fraction (A) of plantain, compared with ~ 38 – 40% in other forages. The proportion of soluble CP was unaffected by N rate in lucerne herbage, but in chicory soluble CP was greater at higher rates of N applied (200 and 350N) compared with lesser rates (0 and 100N) (Table 5.6). The opposite trend was observed in plantain and ryegrass CP, where solubility was reduced by 35 and 10% at 350N respectively. Total potential degrability (P) of lucerne and plantain CP was unchanged by N fertiliser rate, but was increased at the greater N rates in both chicory and ryegrass herbage. The rate of degradation (k) was unaffected by N fertiliser rate in non-legume species, but was reduced by at least half in fertilised lucerne compared to unfertilised lucerne herbage (k 0.05 vs. 0.20 %/h; $P = 0.011$). The effects of N rate on solubility of CP, and potential degradability in chicory and ryegrass herbage resulted in differences ($P < 0.001$) in the overall degradation curves for these species (Figure 5.7).

Table 5.5 Effect of rate of nitrogen (N) fertilisation on the soluble (A) and degradable insoluble (B) fractions (%) and rate of degradation of the B fraction of *dry matter* in chicory, lucerne, plantain and perennial ryegrass herbage collected in autumn.

	N fertiliser application rate (kg N/ha)					
	0	100	200	350	SE	*p* - Value
Chicory						
A	34.6[c]	36.9[b]	37.3[b]	38.8[a]	0.18	0.045
B	59.5	56.6	58.0	56.5	1.08	0.892
P	94.1	93.5	95.3	95.3	1.12	0.246
U	5.9	6.5	4.7	4.7	1.12	0.317
k	0.16	0.14	0.14	0.11	0.02	0.810
Lucerne						
A	27.7	31.3	29.5	24.5	0.18	0.205
B	54.1[a]	49.9[b]	55.9[a]	57.1[a]	1.08	0.031
P	81.9	81.3	85.4	81.6	1.12	0.465
U	18.1	18.7	14.6	18.4	1.12	0.433
k	0.12	0.13	0.15	0.12	0.02	0.261
Plantain						
A	33.7[a]	33.6[a]	32.0[b]	32.0[b]	0.18	0.012
B	56.6	55.1	57.3	58.1	1.08	0.855
P	90.3	88.8	89.3	90.2	1.12	0.835
U	9.7	11.2	10.7	9.8	1.12	0.693
k	0.14	0.17	0.13	0.17	0.02	0.554
Perennial ryegrass						
A	33.7[c]	35.7[b]	37.5[a]	37.0[a]	0.18	< 0.001
B	58.6	59.1	54.6	57.7	1.08	0.101
P	92.3	94.8	92.1	94.8	1.12	0.249
U	7.7	5.2	7.9	5.2	1.12	0.752
k	0.04	0.04	0.05	0.03	0.02	0.640

Note: Means in rows sharing the same lower case superscript are not significantly different

Table 5.6 Effect of rate of nitrogen (N) fertilisation on the soluble (A) and degradable insoluble (B) fractions and rate of degradation of the B fraction of *crude protein* in chicory, lucerne, plantain, and perennial ryegrass herbage collected in autumn.

	N fertiliser application rate (kg N/ha)					
	0	100	200	350	SE	*p* - Value
Chicory						
A	30.5^{d}	33.7^{c}	45.3^{a}	42.9^{b}	0.17	< 0.001
B	63.3^{a}	59.4b	51.7^{c}	52.5^{c}	0.74	0.024
P	93.8^{b}	93.1^{b}	97.0^{a}	95.4^{ab}	0.79	< 0.001
U	6.2^{a}	6.9^{a}	3.0^{b}	4.6^{ab}	0.79	< 0.001
k	0.20	0.16	0.16	0.14	0.02	0.525
Lucerne						
A	40.3	42.2	39.2	40.4	0.17	0.693
B	52.6	52.4	54.9	55.3	0.74	0.865
P	92.9	94.5	94.1	95.7	0.79	
U	7.1	5.5	5.9	4.3	0.79	
k	0.20^{a}	0.10^{b}	0.06^{b}	0.05^{b}	0.02	< 0.001
Plantain						
A	18.2^{a}	12.2^{b}	12.4^{b}	12.5^{b}	0.17	< 0.001
B	69.9^{b}	76.2^{a}	76.8^{a}	76.4^{a}	0.74	0.001
P	88.2	88.4	89.2	88.9	0.79	0.258
U	11.8	11.6	10.8	11.1	0.79	0.371
k	0.20	0.19	0.16	0.22	0.02	0.526
Perennial ryegrass						
A	38.0^{a}	38.5^{a}	38.9^{a}	34.8^{b}	0.17	< 0.001
B	49.4^{b}	49.7^{b}	51.2^{b}	58.1^{a}	0.74	0.045
P	87.4^{c}	88.2^{bc}	90.1^{ab}	92.9^{a}	0.79	< 0.001
U	12.6^{a}	11.8^{ab}	8.9^{bc}	7.1^{c}	0.79	< 0.001
k	0.11	0.15	0.14	0.11	0.02	0.453

Note: Means in rows sharing the same lower case superscript are not significantly different at the 5% level.

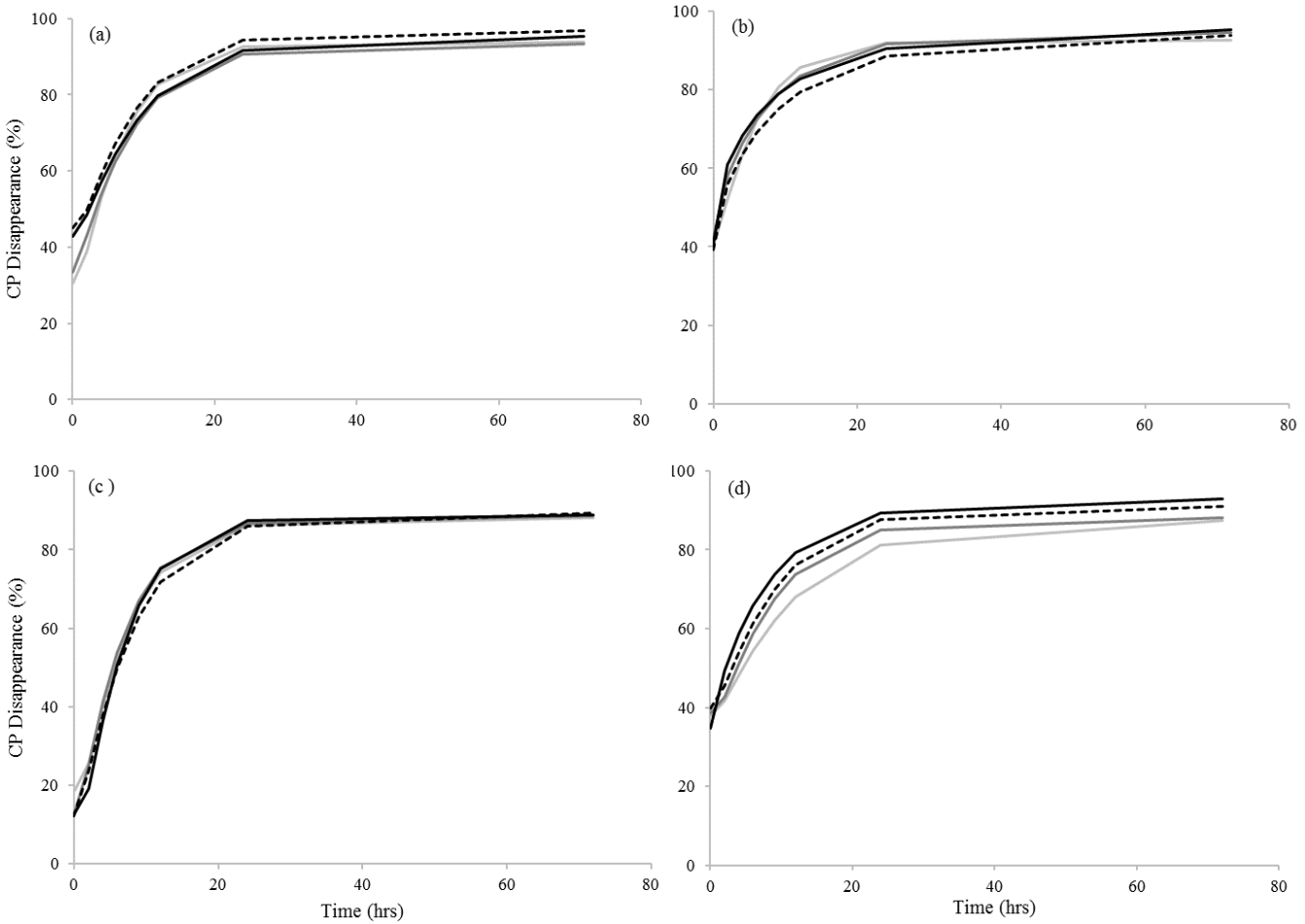


Figure 5.7 *In sacco* degradation of crude protein (CP) from macerated herbage of chicory (a), lucerne (b), plantain (c) and ryegrass (d) receiving nitrogen fertiliser application rates of 0 (light grey), 100 (dark grey), 200 (dashed black line) and 350 (black line) kg N/ha/y.

5.4 Discussion

5.4.1 Herbage physical characteristics

This study demonstrated that the DMY of chicory, plantain and ryegrass increased with the addition of N fertiliser, while lucerne was unaffected. The yield responses of the non-legume forages ranged from 273 – 996 kg DM/ha in autumn and 446 – 2273 kg DM/ha in spring as N fertiliser rate increased from 0 to 350N (Figure 5.2), and these responses were in the range of published data (Belesky et al., 1999; Binnie et al., 2001; Brown et al., 2005; Martin et al., 2017). The absence of a yield response from lucerne to fertiliser N agrees with other studies with legumes (Harris and Clark, 1996; Martin et al., 2017) and reflects their ability to fix their own N. The addition of N fertiliser at 200N at least doubled yields of chicory, plantain, and ryegrass, but responses were much greater in spring than in autumn (Figure 5.2). The strong response of the non-legume species evaluated in this study to N fertiliser application may reflect the soil N status, and the absence of recycled N from excreta as the experiment was managed under and also in the absence of irrigation (Pembleton et al., 2013).

Unlike ryegrass that attained maximum yield and maximum leaf extension at N fertiliser rates of 200N, plantain continued to expand its leaf length and accumulate yield in both seasons as did chicory in spring (Figure 5.2). Others have reported positive linear responses in DM yield from chicory and plantain receiving N rates up to 480 kg/ha/y (Belesky et al., 2000; Martin et al., 2017). Dose response curves (e.g. Figure 5.1 and the data in Figure 5.2) can indicate the optimal fertiliser rate for DM yield of a given species. While this was attained for ryegrass at 200N in the current study, it is apparent that there is potential for further increases in yield from chicory and plantain beyond application rates used here. However, the magnitude of the response to incremental addition of N declined in chicory, but not plantain when rates exceeded 200N. A similar observation was made by Clark et al. (1990) investigating the DM yield response of chicory swards to increasing N fertiliser rate. It would be prudent to investigate whether the additional yield justifies the financial and environmental costs of the additional N fertiliser applied.

Progressively increasing the rate of N applied from 0 – 350N to chicory, plantain and ryegrass swards increased leaf length (i.e. sward height) by 60 – 140% with the greatest increases observed in chicory swards. The width of chicory and plantain leaves also increased with increasing N rate, but leaf width expansion was not observed in ryegrass or lucerne leaves. Thus, the addition of N has a marked effect on the physical presentation of non-legume swards. Furthermore, ryegrass herbage became weaker (~ 42% less force, N/mm^2, required to punch) as N application rate increased, and was associated with declining DM and NDF concentration (section 4.4.2). There are potential farm management implications for the altered physical presentation of swards. For example, current recommendations for grazing ryegrass favour using the number of live leaves per tiller to determine grazing interval (Fulkerson and Slack, 1995). Since leaf emergence is not affected by N supply (Davies, 1988), at the target 2.5 leaf stage ryegrass swards may be deemed physiologically ready for grazing under low fertiliser N (i.e. 0 – 100N) but the associated low sward height and increased herbage toughness may pose a physical impediment to grazing (Weston, 1996; Delagarde et al., 1997). Increasing the physical hindrance to grazing can reduce feed intake of cows (Laca et al., 1992) which could lead to undesirable reductions in productivity. Conversely, the greater leaf extension of chicory and plantain observed under higher rates of N fertiliser could facilitate shorter harvest intervals or more grazing events in a season when timing of harvest is dictated by sward height, as recommended by Lee et al. (2015a). Therefore, the N fertiliser application rate could affect both DM and N intake through herbage growth and sward morphology, and has implications for rotation length of non-legumes on farm.

5.4.2 Herbage chemical composition

Increasing N fertiliser rate applied to the four forages in this study influenced herbage total N content and N fractions but had little effect on carbohydrate fractions. Consistent with the literature, ryegrass, chicory, and plantain herbage increased in total CP concentration when fertiliser N was applied at greater rates (200 – 350N vs. 0 – 100N) (Table 5.1) (Van Vuuren et al., 1991; Valk et al., 2000; Binnie et al., 2001; Loaiza et al., 2016; Martin et al., 2017). The greatest increases in CP concentration coincided with either maximum DM yield, as occurred in ryegrass, or when the rate of DM accumulation had begun to decline, as observed in plantain. Unlike plantain and ryegrass, the total CP

concentration in chicory herbage did not continue to increase when N application increased from 200 – 350N. Similarly, Martin et. al. (2017) observed only a small (<5 %) increase in CP concentration of chicory herbage with increasing fertiliser N application. It is probable that chicory utilised this additional N in spring for increased DMY and/or leaf expansion (Figure 5.2) or has less capacity to take up N from the soil when maximal growth is achieved compared to the plantain and ryegrass.

Herbage nitrate concentration increased with increasing N application rate in all forages in both seasons ($R^2 > 0.7$), reflecting previous studies on the effect of N fertiliser on composition of grass herbage (Goswami and Willcox, 1969; Wilman and Wright, 1986). These data agree with the conclusion of Whitehead (2000) that forages continue to accumulate (and store) N beyond that which is needed for growth. Increasing nitrate concentration increases herbage CP solubility because nitrate molecules are small and are stored in the cytoplasm of the plant cell (section 4.4.1) and are readily released when plant cells are ruptured. As discussed in earlier chapters, rapid and extensive release of CP could result in N supply in excess of rumen microbial capacity for utilisation, depending on the amount of available carbohydrate, but excessive nitrate is toxic and can lead to livestock death when concentrations exceed 0.3 g/100 g DM (Bolan and Kemp, 2003). Application of N fertiliser to lucerne swards increased nitrate concentrations to potentially toxic levels in both seasons (Table 5.3). Excess N is ultimately excreted (Beever and Reynolds, 1994; Castillo et al., 2001a; Phuong et al., 2013) and a potential source of N leaching. Van Vuuren et al. (1991) suggested that dietary CP concentrations exceeding 200 g/kg DM will result in substantial losses of N in urine. This value was exceeded in lucerne herbage in both seasons at all N fertiliser rates, indicating that the N excretion from cows fed diets of lucerne will be high, and may be thus unsuitable as a sole diet for grazing livestock. Autumn ryegrass herbage receiving 350N also exceeded this CP concentration threshold. Autumn is a vulnerable period in terms of N leaching, when cooling soil temperatures mean reduced N uptake from soil by plants and increased rainfall can wash N from the root zone into groundwater. Therefore, applications of N fertiliser to ryegrass should not exceed equivalent rates of 200N in autumn, as this is likely to result in more dietary CP being lost in urine.

Increasing herbage N associated with fibre (NDIN) has potential to reduce the amount of N excreted in urine, because NDIN is less susceptible to degradation in the rumen (Valk et al., 1996) and could increase partitioning of excess N into faeces rather than urine (Bryant et al., 2012). Greater partitioning to faeces rather than urine will reduce losses to leaching because N from faeces is degraded more slowly and to a lesser extent in the soil compared to that from urine (Lockyer and Whitehead, 1990; Jarvis, 1993). Differences in NDIN concentration were evident between forages in both seasons, approximately twice as much NDIN was present in plantain and ryegrass herbage compared with lucerne and chicory herbage ($P < 0.001$). However, the effect of N fertiliser rate on NDIN concentration within forage was not consistent, with no effect in autumn and an interaction in spring (P species × N rate = 0.019). In spring, applying fertiliser N at a rate of 350N increased NDIN concentration in chicory and ryegrass relative to 0N (0.65 vs. 0.10 and 0.69 vs. 0.45 g/100 g respectively), but additional N application reduced NIDN concentration in plantain and lucerne compared with 0N swards (Table 5.3). The increase in NDIN in ryegrass at 350N is similar to the findings of Goswami and Wilcox (1969) with ryegrass, where increases were only observed at relatively high rates of N fertiliser application (250 kg N/ha). There is no known published data concerning chicory, plantain or lucerne. The lack of consistent responses suggests there is little potential for increasing NDIN, and altering N partitioning to faeces by altering N fertiliser application rate, especially as NDIN comprised less than 1% of DM in the forages.

Carbohydrates are the main source of energy for ruminants (NRC, 2001). In a review of the literature, Peyraud and Astigarraga (1998) concluded that WSC concentration in forage grass species decreases with increasing fertiliser applied, but this is not supported by the findings of this study. In fact, the WSC concentration increased in autumn chicory herbage (8.7 – 13.9 g/100 g DM) with increasing N fertiliser. When NSC was calculated, there were no effects of N rate on NSC concentration in chicory, and although values declined in ryegrass herbage with increasing N fertiliser (24.4 – 17.9 g/100g DM) in autumn, there were no effects in spring. The WSC assay used in this study underestimates the concentration of fermentable carbohydrates concentration in forages, and alternative assays must be considered for future investigations.

Fibre (NDF) concentration was unaffected by fertiliser N application rate in all instances except for ryegrass in spring where increasing N rate reduced NDF concentration by 15%. The high fibre content of less fertilised ryegrass herbage may be associated with a higher content of dead material and stem. The literature reports inconsistent relationships between rate of N fertiliser application and NDF, with some researchers demonstrating increased fibre concentration at reduced N fertiliser application (Van Vuuren et al., 1991; Loaiza et al., 2016) while others have shown no effect of fertiliser N on herbage fibre concentration (Valk et al., 2000; Binnie et al., 2001). These findings, and limited published research on the effect of N fertiliser on the carbohydrate fractions of other common species suggest further research is required to understand cause and effect relationships.

It has been proposed that the NUE of a forage species can be improved by altering the amount of soluble carbohydrate relative to nitrogen in herbage (Phuong et al., 2013). Parsons et al. (2011) showed that when the ratio of WSC: CP exceeds 0.7, the amount of N consumed by cattle that is then excreted in the urine declines, and does so progressively as the ratio increases, suggesting improved utilisation of N at greater ratios. The high CP concentration (> 20 g/100 g DM) and low WSC concentration of lucerne herbage meant the ratio never exceeded the threshold, and again indicates that N in lucerne herbage would be inefficiently utilised, resulting in a substantial loss of N to urine. Because CP concentration in plantain and ryegrass herbage tended to increase with increasing fertiliser N application, the WSC: CP ratio declined with increasing fertiliser, nearing the threshold in plantain herbage in both seasons and falling below it in autumn ryegrass when fertilised at 350N. Chicory herbage was often below this threshold in autumn and below that of ryegrass, which would suggest that chicory is a low NUE feed. However, this is not supported by the literature, as studies have shown that cattle fed diets containing chicory excrete urine with lesser N concentrations than those fed ryegrass (Woodward et al., 2012; Minneé et al., 2017). When the NSC: CP ratio was calculated, the trends in ratio with N fertiliser remain, but rather this method suggests chicory and plantain would be more NUE than ryegrass (Figure 5.3) and is more in agreement with the literature. While both methods of calculating the readily fermentable carbohydrates (WSC and NFC) have limitations, both illustrate similar trends and notably a decline in the ratio, suggesting a decline in NUE, with increased fertiliser

N application in plantain and ryegrass herbage. Furthermore, the ratio declines steeply beyond 200N, therefore when considering NUE, 200N ought to be the upper limit of fertiliser N application for plantain and ryegrass swards.

5.4.3 Herbage comminution and release of nutrients

In the context of this study, N application rate had little effect on the extent of comminution of herbage from the four forages as evaluated by the mechanical maceration technique. The data supports the findings of the previous experiment (Chapter 4) which showed that ryegrass was the only one of five forages evaluated where significant variation within forage in the extent of comminution (as determined by particle size distribution, PSD) was observed. Unfertilised spring ryegrass was reduced in particle size to a lesser extent when compared to fertilised ryegrass herbage and this was associated with a greater fibre concentration, supporting the findings in section 4.3.3. Unfertilised ryegrass was more resistant to comminution which may reduce feed intake.

The study in Chapter 4 reported that the release of CP by maceration varied between forages, and this tended to be positively associated with concentrations of CP and NPN in the herbage. The current study confirmed those findings, with ryegrass and lucerne tending to release greater amounts of CP than chicory and plantain (Figure 5.6), and the amount released was correlated with total CP and nitrate concentrations in herbage (Appendix Table B4). The relationship was more prevalent in autumn, where release of CP was positively associated with CP and NPN concentration in chicory, plantain and ryegrass, but in spring the relationship was only observed in ryegrass. It may be that in spring, when growth rates were greater compared with autumn, there was increased demand for N for growth and therefore less stored N. Since the relationship between N fertiliser rate and CP concentration was linear for ryegrass in both seasons and for plantain in autumn, the release of CP in response to N fertiliser was also linear, i.e. increasing fertiliser N application increased release of CP. It was discussed previously that the amount of CP released (or 'solubility' of CP) is important as this can influence NUE. Therefore, considering the strong associations between herbage CP release and CP concentration and with N fertiliser in autumn, particular care should be taken with the application of fertiliser at this time. The findings of this study suggest a limit of 200N to plantain, chicory, and ryegrass swards.

5.4.4 Dry matter and crude protein degradation

Increasing the rate of N fertiliser applied to the four forages in this study had small effects on the solubility of DM following maceration, but did not affect the rate of DM degradation (k) or potential degrability (P) of the herbage (Table 5.5). Reports of improved degrability associated with increased N fertiliser application often involved N application rates exceeding 480 kg N/ha/y, well above rates used in this study, and have attributed improved degradation to lower fibre concentrations in herbage (Van Vuuren et al., 1991; Salaun et al., 1999; Valk et al., 2000). Material used in the *in sacco* study was obtained in autumn where fertiliser treatment had no effect on herbage NDF concentration. If spring ryegrass herbage was used instead, where fertiliser did reduce herbage NDF, toughness, and increase extent of fragmentation, differences in DM degradation may have occurred. Greater feed degradability can increase the rate of feed clearance from the rumen, and in turn livestock DM intake and productivity (Allen, 2014) but these data do not suggest that degradability of forages DM is improved by the addition of N fertiliser in autumn.

The *in sacco* degradation of CP can indicate the pattern of herbage N supply to the rumen microflora, which has implications for NUE. The effects of rate of N application on CP degradation were inconsistent between forages, in line with contrast reports in the literature concerning the effect of N fertiliser on herbage CP degradation. Solubility of chicory CP increased by 25% at greater N rates (200 – 350N vs. 0 – 100N), but the opposite was observed in plantain and ryegrass herbage. However, most often no change (Salaun et al., 1999), or an increase in solubility with increasing N, is observed (Van Vuuren et al., 1991; Valk et al., 1996), though the latter investigated a wide range of N application rates (0 - 700 kg N/ha/y). In this study, the greater quantity of CP lost from more highly fertilised chicory swards was likely associated with the greater CP content of these herbages, in agreement with the trends observed for CP loss during maceration (section 5.3.2 and 5.4.3). In ryegrass, the decrease in soluble CP released from herbage by maceration coincided with measured increased herbage toughness under low N fertiliser rates, potentially meaning the mechanical macerator applied more force to fragment the material in preparation for incubation. This could have resulted in greater crushing and rupture of plant cells leading to greater release of CP, although this proposition is not supported by the CP release data

(section 5.3.3) that showed an opposite trend of increasing CP loss with increasing N rate. The effects are unclear, and furthermore there was no apparent explanation for the greater solubility of unfertilised compared to fertilised plantain herbage. In terms of improving NUE, lesser soluble CP might promote greater utilisation through a slower release, resulting in a lower rumen ammonia concentration, in which case these data may provide support to the suggestion that chicory ought not receive N fertiliser rates exceeding 200N.

The small increase in theoretical rumen degrability (P) of CP from chicory and ryegrass herbage fertilised with higher rates of N agrees with observations by Salaun et. al. (1999) who investigated ryegrass. However, others have reported no or inconsistent effects (Van Vuuren et al., 1991; Valk et al., 2000), as was observed for plantain herbage in the present study. These data suggest that the effect of N fertiliser rate on DM and CP degrability is often small and inconsistent (partly due to variation in technique), that greater variation occurs between forages. Therefore, it can be concluded that manipulating fertiliser N application in autumn to manage degradation and NUE will have limited effect.

5.5 Conclusions

The aim of this study was to evaluate the effect of increasing fertiliser N input on four common forage species and explore whether the release of crude protein from herbage could be manipulated through varying N application rate. Increasing fertiliser N rate applied to chicory, plantain, and ryegrass, increased leaf extension, crude protein and NPN concentrations in herbage, and these effects both increased CP release with maceration, and also reduced the ratio of NSC: CP. These results, along with the decline in DM yield in response to fertiliser application exceeding 200N, indicates this should be the upper limit of N application for these forages. Conversely, very low or no N fertiliser applied to ryegrass swards increases sward toughness and reduces sward height (leaf length) as well as DM production, all of which would negatively impact the grazing, intake of and production from livestock.

The findings of this study also confirm there is no benefit in applying N to lucerne swards, in fact, it may have a negative effect in terms of increasing the amount of NPN compounds in the herbage and

reducing NDIN which would increase solubility of the N and likely reduce NUE. For this reason, care should also be taken when applying N fertiliser to mixed swards containing lucerne.

6

Characteristics of ryegrass, lucerne or chicory boli swallowed by dairy cows

6.1 Introduction

Although the chemical composition of herbage determines how much of a given nutrient is consumed, this measure does not describe the pattern of nutrient availability during digestion. Ingestive mastication (mastication) is the first step in digestion, and while there is a significant body of research concerning the ruminal digestion of herbage, less is known about the degradation of herbage during mastication. For cows consuming forage, mastication is particularly important for disrupting the outer cuticle which is highly resistant to microbial degradation enabling microbial access to internal degradable plant material (Cheng et al., 1980).

A second function of mastication is to reduce the size of feed particles, to manipulate the feed into a bolus to facilitate swallowing, and to increase surface area available for microbial adhesion and digestion (Ulyatt et al., 1986). The contribution of ingestive mastication to herbage particle size reduction is usually less than that of rumination, but can reduce 15 – 60% of ingested DM (by weight) (Pond et al., 1984b; McLeod and Minson, 1988; Waghorn et al., 1989), to particles of a size able to pass from the rumen (Ulyatt et al., 1986).

The third function of mastication is cell rupture and release of cell nutrients. Waghorn and Clark (2004) reported 50 – 80% of plant cells in fresh forages were ruptured through mastication in ruminants. Most plant nitrogen and all non-structural carbohydrates (NSC) are stored within the plant cell (Sanderson and Wedin, 1989; Acosta et al., 2007), therefore the extent and pattern of cell rupture and subsequent release of nutrients will impact on their delivery to the rumen microflora and host. Studies investigating nutrient release from temperate grass and clover forage masticated by dairy cows suggest 20 – 30% of intracellular nitrogen and 30 – 50% of NSC is released during ingestion (Boudon and Peyraud, 2001; Boudon et al., 2006; Acosta et al., 2007). Differing physical attributes and chemical composition of

forages are likely to affect the extent of comminution and nutrient release. However, no clear relationship between nutrient release and forage physical or chemical characteristics were reported in previous studies. Further investigation across physically or chemically divergent species to determine which factors influence comminution and nutrient release during mastication is required to improve understanding of forage digestion.

The rate and extent of nutrient delivery to the rumen is relevant to livestock production systems as this can influence efficiency of forage utilisation (Huntington and Archibeque, 2000; Phuong et al., 2013). In forage species-based systems, energy is usually the most limiting nutrient for ruminant production, but N is often in excess of requirements (Brookes and Nicol, 2007). Excess dietary N is excreted in urine and is a source of environmental pollution, as atmospheric ammonia and nitrate in groundwater and as nitrous oxide to the atmosphere (section 2.2) (Tamminga, 1992a; Johnson et al., 2005). Recently, Minneé et al. (2017) showed that cows fed chicory and plantain had lower concentrations of rumen ammonia and N in urine compared to cows fed ryegrass. Increasing our knowledge of how different forages are processed during mastication and the extent of nutrient release may improve our understanding of why such differences occur, and how nutrients are utilised.

The objective of this study was to investigate the effects of mastication by dairy cows fed three physically and chemically contrasting forages on boli characteristics, extent of comminution, cell content release and subsequent degradation.

6.2 Material and methods

6.2.1 Experimental treatments and animals

The experiment compared the characteristics of ingested boli, and nutrient release from herbage of three forages: perennial ryegrass (ryegrass; *Lolium perenne* L. cv. One50 with AR37 endophyte), chicory (*Cichorium intybus* cv. Choice) and lucerne (*Medicago sativa* cv. Torlesse) fed to mature dairy cows. Experimental design was two 3 × 3 Latin squares with three forages and three measurement periods of one day each. A total of six non-lactating, pregnant, multiparous Holstein-Friesian × Jersey crossbred cows were used in the experiment which was conducted over 10 days in May 2015 in Hamilton, New

Zealand (37°76'S, 175°36'E, 40 m a.s.l.). The first Latin square was applied to three mature (10.1 ± 0.61 years old; BW 631 ± 64 kg) cows and the second to three younger (4.8 ± 0.02 years; BW 505 ± 19 kg) cows. The cows were fitted with a large ruminal cannula (i.d., 125 mm), and were randomly allocated one forage each day.

Approval for this experiment was granted by the Ruakura Animal Ethics Committee, application No.: 13556 (Hamilton, New Zealand).

6.2.2 Sward management and animal feeding

The forages were sown in October 2014, received 200 kg N/ha/year in 10 applications of 20 kg N/ha as SustaiN™ (N46; Ballance Agri-nutrients, Tauranga) and were grown without irrigation. The stage of maturity of the swards during the experiment was approximately 2.75 leaves per tiller for ryegrass, 35 cm sward height for chicory, and early-bud stage (44 days of regrowth) for lucerne. Fresh herbage was cut and collected each day at approximately 0800 h using a Jenquip® forage harvester (NZ Agriworks Ltd, Feilding, New Zealand), set to cut at approximately 50% of the mean sward height for each forage to simulate the bite depth of the first grazing bout of dairy cows on fresh pasture (Gregorini et al., 2017). Cut heights were 10, 20 and 15 cm for ryegrass, chicory and lucerne, respectively.

The afternoon before sampling (approximately 1700 h), cows were removed from grazing perennial ryegrass/white clover swards and fasted overnight (approximately 16 h) with unrestricted access to water. At 0900 h cows were moved to individual tie-stalls with rubber mats, open ventilation, and access to water. Their rumens were partially emptied by hand via the cannula, enabling access to the cardia. Rumen contents were stored in covered bins to maintain temperature. Following partial rumen emptying, 11 kg of fresh herbage, was offered to each cow. As the cows ate, boli were collected and the duration of eating recorded. Any feed not eaten after 30 minutes was weighed to calculate herbage intake and rate (g DM/min).

6.2.3 Sampling and measurements on the ingested boli

Methodology for boli collection was based on that described by Acosta et al. (2007) and Boudon et al. (2006). Ingestive boli were sampled three times during the first meal of the day: at the beginning, near

the middle and at the end. At each sampling time, the first bolus collected was discarded, then the next ten consecutive boli were collected from each cow for measurement. Individual boli were firm enough to be removed intact by hand. Plate 6.1. shows individual boli collected and placed in a tared container for weighing. Each bolus was weighed, then all ten boli from each cow were pooled to generate a composite sample for each sampling time. After collection from the final sampling time (approximately 30 minutes after commencement), the stored rumen contents were returned to the rumen and cows were released to graze perennial ryegrass/clover swards.

Plate 6.1. Collected boli in tared sample trays, illustrating their intact form.

The composite sample of boli was gently mixed by hand. From this, two subsamples of 100 ± 5 g were weighed fresh then dried in a forced-draught oven at 95ºC for 48 h before reweighing to determine dry

matter (DM) content. Herbage and saliva content of the boli, and the amount of saliva added per 100g of herbage was calculated using the equations of Reid et al. (1962):

$$Herbage\ content\ of\ bolus\ (F) = (\frac{b - (y\ \times Bw)}{X - Y})$$

Equation 6.1

$$Saliva\ content\ of\ bolus\ = Bw - F$$

Equation 6.2

Where:

b = *dry weight of the boli (g)*
Bw = *wet weight of the boli (g)*
X = *dry matter content of the herbage (g/100 g)*
Y = *dry matter content of the saliva (g/100 g)*

A value of 0.9 g/100 g was used for the DM content of saliva (Y), based on Hart (1983).

A 150 ± 5 g FW subsample was taken for calculation of nutrients released from feed during mastication, using the method described by Boudon and Peyraud (2001). The sub-sample was placed on cheesecloth and rinsed with 3 litres of water by gentle stirring using a spatula to remove released nutrients and saliva. The rinsed residues were freeze-dried and ground in a mill to pass a 1 mm sieve (Christy & Norris Mill, United Kingdom), then analysed to determine total N, water soluble carbohydrate concentration (WSC) and NDF in the DM. Methodology for chemical analyses is detailed in section 4.2.3. Nutrient release was calculated as the difference between the quantity in the herbage and the quantity remaining in the bolus after rinsing. The calculation of Boudon and Peyraud (2001) assumes no loss of NDF during rinsing, and the loss of N is calculated as follows:

$$N\ released = 1 - \left(\frac{N\ boli\ \times\ NDF\ feed}{N\ feed\ \times\ NDF\ boli}\right) \times\ 100$$

Equation 6.3

Particle size distribution (PSD) of the boli was measured using a wet sieving apparatus (Turner and Newell Ltd) with 4 counter-rotating sieves, positioned above a stationary (0.075 mm) sieve. Sieve sizes (as the length of the size of the square hole) were 4, 2, 1, 0.5 and 0.075 mm. Subsamples of 25 g (fresh weight) were placed on the top sieve, then 1500 ml of water recirculated through all sieves at 4

litres/minute for 5 minutes. Material retained on each sieve was transferred on to weighed filter papers then oven-dried at 60ºC for 48 h before weighing to determine the distribution of DM between size fractions (Plate 6.2). The soluble fraction was defined as the DM passing the 0.075 mm sieve, and was calculated as the difference between the total DM sieved and the sum of the DM retained on the five sieves. The lengths of chicory and perennial ryegrass leaves and lucerne stems that were retained on the top sieve were measured with a ruler to the nearest mm (Plate 6.3). All boli samples were measured in duplicate.

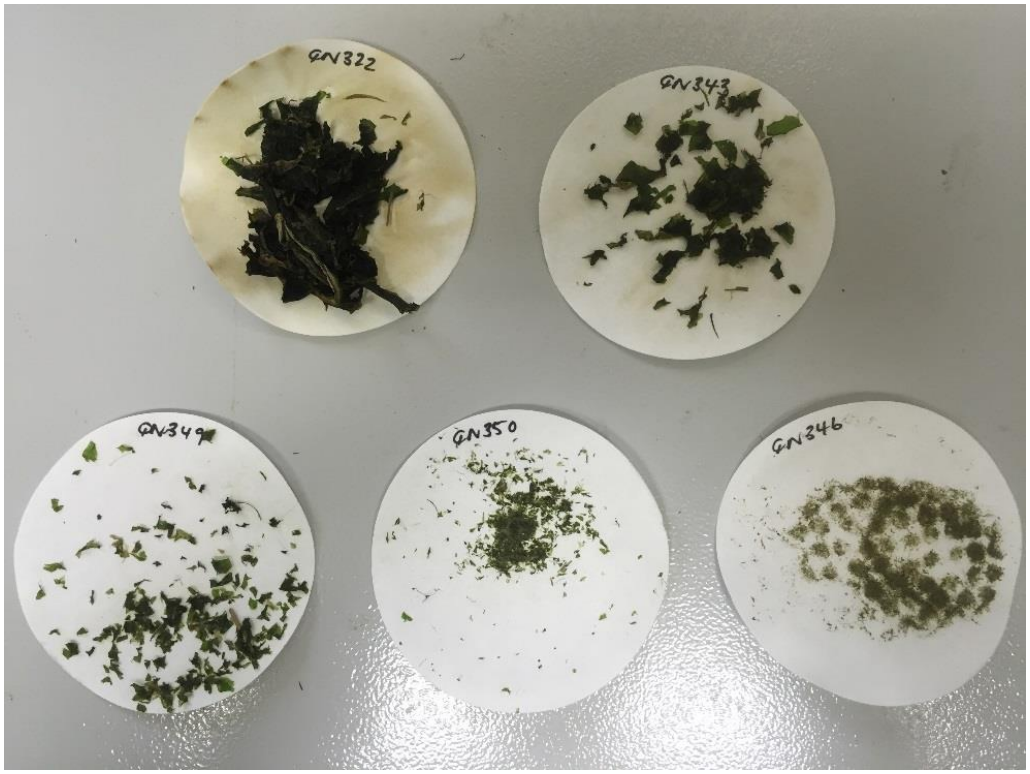


Plate 6.2. Material retained on each sieve from a chicory boli after drying.

Plate 6.3. An example of wet-sieved material from the top sieve (4 mm) spread out for fragment length measurement.

Kinetics of dry matter (DM) and nitrogen (N) degradation of the boli were measured in four fistulated cattle by *in sacco* incubation over 72 h as described in section 5.2.4. Two mature and two younger cows were selected (balanced for BW) from the six cows used for boli collection, and grazed a common allowance of perennial ryegrass/white clover herbage. Boli material used was from the middle sampling during the meal and pooled across all cows for each forage. The proportions of soluble (A) and degradable insoluble (B) and the fractional degradation rate (k) of the B fraction were estimated for DM and CP. Briefly, approximately 35, 33 and 37 g fresh weight of boli material from ryegrass, lucerne and chicory respectively (to achieve ~ 3 g DM) were weighed into 100 x 100 mm Dacron bags (mean pore size 35 µm; Ankom Technology, USA). Each bag was sealed and stored at -20°C. Approximately 15 minutes before incubation, bags were thawed and then seven bags of each feed type were placed in the rumen of each cow. One bag of each feed was removed from the rumen after 2, 4, 6, 9, 12, 24 and 72 h. One additional bag of each forage type that was not placed in the rumen (0 h; pre-degradation sample) and the rumen-incubated bags were hand-rinsed in cold water until no further colour appeared. Bags

were dried at 60°C for 48 h then weighed to calculate DM loss. Residue from the bags was ground to pass through a 1 mm sieve (Thomas Wiley Mill, USA) for estimation of N content by near infrared spectroscopy (NIRS; Massey University, Palmerston North, New Zealand). NIRS spectra were collected on all samples using a multi-purpose analyser 0172.04 with Spectroscopy Software OPUS 7.0 (Bruker Optic GMBH, Madison, Wisconsin, USA). NIRS calibrations for crude protein (CP) concentration are based on N concentration data determined using a Carlo Erba NA1500 elemental N analyser, × 6.25 (AOAC 1990) from a large database of samples (Corson et al., 1999). Standard errors of prediction for CP were 1.13% of DM, with resultant correlation (R^2) of 0.97.

6.2.4 Sward characterisation

On each day of boli collection, 2 kg subsamples from the forages offered were collected. Herbage DM content was determined by weighing and oven-drying triplicate samples of 200 ± 5 g wet weight at 95°C for 48 h. A further sub-sample (200 g) was frozen at -20°C, freeze-dried and ground to pass through a 1-mm sieve (Christy & Norris Mill, United Kingdom) for chemical analysis. Botanical composition of the herbage was determined by hand-sorting sub-samples (200 g) into green leaf, dead leaf, stem, petiole, and unsown species. Sorted samples were dried in a forced-draught oven at 95°C for 48 h to determine composition on a dry weight basis. For morphological measurement, 100 pieces of each feed were randomly sampled and the length of the stem, and length and width of the leaf blade measured using a ruler, or digital callipers where the leaf or stem was less than 20 mm in size (Mitutoyo digimatic CD-8"CS, Mitutoyo Corp, Japan). Two methods (detailed in section 3.3) were used to assess the forages' biomechanical properties. The first method determined the resistance of plant material to fracture by measuring the force (Newtons/mm^2) required to punch a 2 mm diameter hole in the material using a digital force gauge (DS2 Digital force gauge, Imada Inc., Japan). The force to punch herbage was estimated from a weighted average of the force to punch each component (i.e. leaf or stem) and the proportion that component contributes to the herbage. The second method measured the amount of energy required to mechanically macerate the herbage using a Kreft Compact mincer fitted with a sieve plate with 12 mm holes (Kreft, Germany) to provide a particle size distribution similar to rumen digesta (Barrell et al., 2000). To do this, approximately 700 g of frozen herbage was chopped into 20 mm

lengths, then fed into the mincer. A HOBO® analog logger (Onset Computer Corporation, MA, USA) recorded the current (amps) used by the mincer at one second intervals (section 3.3.2).

6.2.5 Chemical analyses

Structural carbohydrates, NDF and ADF were measured using the methods of Van Soest et al., (1991) and method 973.18 (AOAC Int. 2000), respectively, using Whatman 934-AH glass micro-fibre filters with a 1.5 µm particle retention. Fibre recovered from the ADF measurement was used to determine lignin concentration as per Goering and Van Soest (1970). Water soluble carbohydrate concentration was measured colorimetrically by the method of DuBois et al., (1956). Total N concentration was determined by combustion (method 990.03; AOAC Int. 2000) in a LECO FP-528 Nitrogen analyser (Leco, St Joseph, MI, USA). Nitrogen in the NDF residue (NDIN) was measured in the residue from the NDF procedure also using the LECO FP-528 analyser. Concentration of non-protein N components was assessed by the urease and potentiometric methods (941.04 and 986.31; AOAC Int. 2000); and ash by heating 1.5 g of sample to 600°C in porcelain crucibles for 4 h (method 942.05; AOAC Int. 2000).

6.2.6 Statistical analyses

Forage physical and chemical data were analysed as a Latin square by analysis of variance (ANOVA) in SAS version 9.3 (SAS Institute Inc., NC, USA). A mixed models approach to repeated measures ANOVA was used for boli data (Proc Mixed, SAS 9.3). ANOVA was followed by Tukey's t-test for pairwise comparisons. The effect of forage species on the particle size distribution of masticated forage, were analysed by a one-way ANOVA (SAS 9.3). Data were averaged across time collected in the meal and cows to obtain one result per measurement date and forage. Measurement dates were used as replicates.

Relationships between variables were investigated using regression analysis to fit linear equations, first using the mean values for each forage to test for associations between variables using between forage variation, then using individual replicates (days) to test the association within forage. The regression analysis provided slope, SE of the slope, *P*-value and the R^2 coefficient.

6.3 Results

6.3.1 Herbage characteristics and chemical composition

Chicory and ryegrass herbage was vegetative, and predominantly green leaf material (Table 6.1). Lucerne herbage comprised 55% green leaf and 45% stem + petiole (DM basis). The physical and chemical properties of the forages offered are detailed in Table 6.1. Ryegrass and lucerne had similar mean fragment length which was approximately 60 mm shorter than chicory ($P < 0.001$). Herbage dry matter content was least for chicory (76 g/kg) and similar (122 – 127 g/kg) for lucerne and ryegrass ($P < 0.001$). Measures of the biomechanical properties of the forages suggested that lucerne leaves require approximately 1/3 of the force to be fractured compared with chicory and ryegrass ($P < 0.001$), but lucerne stem required 10 × the force for leaves to be fractured. Energy required to mechanically macerate the herbage was greatest for ryegrass at 113 J/g FW, and least for chicory, with lucerne intermediate (22 and 55 J/g FW; $P < 0.001$). Total N concentration was greatest in lucerne, which also contained the most lignin. Water soluble carbohydrate in chicory was nearly twice that of the other two forages. Ryegrass herbage had the greatest cell wall content (423 compared with 201 and 238 g NDF/g DM from chicory and lucerne) and the most fibre-bound protein.

Table 6.1 Physical and chemical characteristics of the chicory, lucerne and perennial ryegrass offered.

	Forage				
	Chicory	Lucerne	P. ryegrass	RMSE	*P*-Value
Mean length of offered forage (mm)	200[a]	147[b]	138[b]	18.0	< 0.001
Mean leaf width (mm)	47[a]	15[b]	3[c]	1.9	< 0.001
Dry matter (DM, g/kg)	76[b]	127[a]	122[a]	3.1	< 0.001
Botanical composition (%)					
Leaf	100[a]	55[b]	97[a]	3.6	< 0.001
Stem	0[b]	45[a]	3[b]	3.8	< 0.001
Punch force (Newtons/mm^2)					
Leaf	0.89[a]	0.32[b]	0.93[a]	0.07	< 0.001
Stem	-	3.4	-	0.50	
Maceration energy					
J/g DM	294[c]	430[b]	915[a]	57.3	< 0.001
J/g FW	22[c]	54[b]	112[a]	6.1	< 0.001
Chemical composition (g/kg DM)					
Total nitrogen (N)	31[c]	49[a]	38[b]	0.31	< 0.001
Urea + ammonia N	0.32[b]	0.44[a]	0.36[ab]	0.12	0.004
Nitrate N	1.6[b]	5.0[a]	1.4[c]	0.80	0.019
Neutral detergent insoluble nitrogen	2.5[b]	1.4[c]	4.3[a]	0.45	< 0.001
Water-soluble carbohydrates	138[a]	69[b]	87[b]	16.7	< 0.001
Neutral detergent fibre	201[c]	238[b]	423[a]	23.7	< 0.001
Acid detergent fibre	135[c]	208[b]	258[a]	20.2	< 0.001
Lignin	28[b]	42[a]	24[b]	6.01	0.001
Ash	163[a]	100[b]	111[b]	4.6	< 0.001

Values within a row with different superscripts differ significantly at $P<0.05$

6.3.2 Characteristics of ingested boli

The boli weight, herbage and saliva content and intake rates of the herbage are presented in Table 6.2. The average fresh weight of the boli did not differ between forages but the quantities of herbage and saliva did. Boli of chicory contained the most herbage material (g fresh weight, FW), while ryegrass boli had the greatest saliva content ($P < 0.01$). Saliva (g) added to ryegrass was 63 g/100 g of herbage, which was 21 and 9 g more saliva than was added to chicory or lucerne, respectively ($P = 0.013$). Intake rate of fresh herbage (FW; g FW/min) was similar between forages, however intake rate of DM was greatest for cows consuming ryegrass (71 g DM/min) compared with chicory or lucerne (47 and 65 g DM/min respectively).

Table 6.2 Characteristics of ingested boli and intake rate of dairy cows fed chicory, lucerne or perennial ryegrass indoors.

	Forage				
	Chicory	Lucerne	P. ryegrass	RMSE	*P*-Value
Fresh weight of boli (g)	171	162	170	16.3	0.605
Dry weight of boli (g)	9.0^{b}	14.0^{a}	13.4^{a}	2.18	< 0.001
Feed content of boli (g)	120.3^{a}	107.2^{b}	104.9^{b}	5.5	0.048
Saliva content of boli (g)	47.6^{b}	54.7^{b}	65.4^{a}	4.0	0.004
Saliva added per 100 g feed (g)[1]	42.4^{b}	54.3^{ab}	63.3^{a}	5.5	0.013
Intake rate (g FW/min)	621	509	579	97.7	0.187
Intake rate (g DM/min)	46.5^{b}	64.7^{ab}	70.7^{a}	10.94	0.032

[1] Calculated according to the formula of Reid et al. (1962). Values within a row with different superscripts differ significantly at P<0.05

Bolus weight and feed content in the boli from cows consuming chicory or ryegrass tended to increase as the meal progressed but not when consuming lucerne (Table 6.3). The amount of saliva in the boli and added to feed (g/100 g feed) was not affected by time within the meal.

Dry weight of the boli and feed content in the boli did not differ between cows and no boli characteristics were affected by the age of the cow (Appendix Table B.6).

Table 6.3 Differences between characteristics of ingested boli from dairy cows fed chicory, lucerne or perennial ryegrass sampled at three times during a meal.

	Time in meal				
	Start	Middle	End	RMSE	*P*-Value
Fresh weight of boli (g)					
Chicory	145.3^{b}	171.3^{a}	187.1^{a}	16.3	<0.001
Lucerne	149.8^{b}	174.3^{a}	161.6^{ab}	16.3	0.047
P. ryegrass	148.0^{b}	169.9^{ab}	192.3^{a}	16.3	<0.001
Dry weight of boli (g)					
Chicory	8.2	9.8	8.9	2.18	0.432
Lucerne	12.8	15.4	13.8	2.18	0.132
P. ryegrass	11.3^{b}	13.3^{ab}	15.4^{a}	2.18	0.013
Feed content of boli (g)					
Chicory	100.5^{b}	122.3^{ab}	138.1^{a}	13.8	<0.001
Lucerne	97.6	118	105.8	13.8	0.051
P. ryegrass	89.2^{b}	104.2^{ab}	121.2^{a}	13.8	0.002
Saliva added per 100 g feed (g)					
Chicory	45.9	42.6	39.4	12.6	0.707
Lucerne	54.0	49.0	60.0	12.6	0.333
P. ryegrass	67.4	63.1	59.2	12.6	0.534

Values within a row with different superscripts differ significantly at $P<0.05$.

6.3.3 Herbage comminution and release of nutrients

The PSD in boli (Table 6.4) show the proportion of large particles (> 4 mm) was greatest in chicory, compared with lucerne and ryegrass (0.64, 0.56 and 0.59, respectively, $P < 0.05$), but was similar in other fractions. There were no differences in the proportion of large (> 4 mm) particles and 'soluble' DM in boli between cows ($P = 0.079$ and $P = 0.345$, respectively).

Further separation of the particles retained on the sieve with 4-mm apertures, based on fragment length (Table 6.4) showed that ryegrass boli had a greater proportion of shorter particles (4 – 40 mm) than chicory or lucerne, which had a similar distribution of particle lengths ($P = 0.015$). Lucerne had the longest median particle length, and ryegrass the shortest. The longest particle length measured in the boli from each forage was a 251 mm lucerne stem, and 193 mm chicory and 212 mm ryegrass leaves. Median particle length did not differ at different time points in the meal ($P > 0.05$). Particle size distribution in boli was not affected by the age of the cow.

There were no relationships between any of the physical or chemical characteristics measured and the extent of herbage comminution (as determined by PSD), when all forages from all sampling dates (n = 18) were included in the analyses. However, with ryegrass, stem content in the herbage and the energy required for maceration explained 23% of the variation in PSD. Where stem content and energy required to macerate increased, the proportion of large particles (> 4mm) in macerated material declined ($P < 0.05$). This relationship was not observed in chicory or lucerne.

Table 6.4 Proportion of size distribution of masticated particles from cows offered chicory, lucerne or perennial ryegrass; and the distribution of particle sizes (mm) of those retained on the top sieve (4 mm).

	Forage				
	Chicory	Lucerne	P. ryegrass	RMSE	*P*-Value
Distribution of particles					
> 4 mm	0.65^{a}	0.56^{b}	0.58^{ab}	0.020	0.0258
1 - 2 mm	0.08^{b}	0.12^{a}	0.13^{a}	0.016	0.007
0.075 – 0.5 mm	0.05^{b}	0.07^{a}	0.05^{b}	0.009	0.030
Soluble (< 0.075 mm)	0.24	0.26	0.24	0.053	0.426
Distribution of large particles > 4 mm					
4 – 40 mm	0.68^{b}	0.66^{b}	0.82^{a}	0.07	0.015
41 – 80 mm	0.26^{a}	0.24^{a}	0.15^{b}	0.05	0.013
81 – 120 mm	0.05^{ab}	0.06^{a}	0.02^{b}	0.02	0.012
> 121 mm	0.03	0.04	0.01	0.036	0.300
Median fragment length of large particles > 4 mm	29.2^{ab}	33.8^{a}	22.4^{b}	6.3	0.044

Values within a row with different superscripts differ significantly at $P<0.05$.

The proportion of herbage WSC released during mastication was greater than that of CP for chicory (0.34 vs 0.15) and ryegrass (0.31 vs. 0.20), but not for lucerne (Table 6.5). Although the proportion of WSC released from chicory (mean = 0.34) was similar to that from ryegrass (0.31), the total release from chicory was double that of ryegrass and lucerne (48 vs 23 and 21 g/ kg DM) because chicory herbage contained more WSC. Nitrogen loss also differed between the forages ($P = 0.015$), with the greatest proportion (0.26) from lucerne. Amount of N released were 5, 12 and 8 g N/kg DM from chicory, lucerne and ryegrass, respectively. When considering intake rate (g DM/min) the WSC release was 2.25, 1.38 and 1.66 g/min from chicory, lucerne and ryegrass, respectively, while N loss was 0.22, 0.54 and 0.79 g/min from respective forages. There was a moderate correlation between the amount of N released during ingestive mastication and the amount of N in the forage ($R^2 = 0.53$, n = 52; $P < 0.001$) but no relationship between WSC concentration and WSC release ($R^2 = 0.17$). In lucerne, cell content release was strongly correlated with the extent of comminution (PSD), where WSC and N release increased with increasing proportion of small particles in masticated material ($R^2 = 0.68$ and 0.70 for WSC and N loss respectively; $P < 0.001$). This relationship was not observed in the other forages.

Table 6.5 Proportion of nutrient release from chicory, lucerne and perennial ryegrass during mastication by dairy cows indoors during three sampling times during one meal and the mean of all times.

	Forage				
	Chicory	Lucerne	Ryegrass	RMSE	*P*-Value
Water-soluble carbohydrate					
Start	0.30^{B}	0.28	0.32	0.027	0.097
Middle	0.35^{aAB}	0.26^{b}	0.31^{ab}	0.028	0.005
End	0.39^{aA}	0.28^{b}	0.31^{b}	0.027	<0.001
Mean	*0.34^{a}*	*0.27^{b}*	*0.31^{a}*	*0.016*	*0.005*
Nitrogen					
Start	0.12^{b}	0.26^{a}	0.19^{ab}	0.021	<0.001
Middle	0.16^{b}	0.25^{a}	0.20^{ab}	0.024	<0.001
End	0.15^{b}	0.27^{a}	0.22^{ab}	0.022	<0.001
Mean	*0.15^{c}*	*0.26^{a}*	*0.20^{b}*	*0.019*	*0.015*

Values within a row with different lowercase superscripts differ significantly at $P<0.05$; values within columns with different uppercase superscripts differ significantly at $P<0.05$

Sampling time in the feeding bout had no effect on the release of N during mastication, or of WSC from lucerne and ryegrass. However, the proportion of WSC released from chicory increased as the meal progressed ($P = 0.001$).

6.3.4 Degradation of dry matter and crude protein in boli

The degradation characteristics of herbage DM and CP are presented in Table 6.6. The amount of solubilised (A fraction) DM was greatest in ryegrass, followed by chicory and then lucerne ($P < 0.001$). By contrast, solubilised CP was greatest in lucerne, which was twice that of chicory and ryegrass which were similar to each other. Potential degradability of DM and CP was least in lucerne, but similar between chicory and ryegrass. But the rate of degradation of both DM and CP was similar across all species ($P > 0.05$).

Table 6.6 Dry matter (DM) and nitrogen (N) degradation characteristics of chicory, lucerne and ryegrass herbage by *in sacco* digestion. Degradation characteristics are defined by soluble (A), degradable insoluble (B), potentially degradable (P), and undegraded (U) fractions, and the degradation rate of the B fraction (k, %/h).

	Forage				
	Chicory	Lucerne	Ryegrass	SE	*p*- Value
DM degradation					
A	0.18^{b}	0.15^{c}	0.22^{a}	0.01	< 0.001
B	0.75^{a}	0.69^{b}	0.69^{b}	0.14	0.008
P	0.93^{a}	0.85^{b}	0.91^{a}	0.14	0.001
U	0.07^{b}	0.16^{a}	0.09^{b}	0.14	0.001
k	0.18	0.16	0.24	0.06	0.612
N degradation					
A	0.06^{b}	0.14^{a}	0.07^{b}	0.02	< 0.001
B	0.88^{a}	0.74^{b}	0.89^{a}	0.06	< 0.001
P	0.94^{a}	0.88^{b}	0.96^{a}	0.06	< 0.001
U	0.06^{b}	0.12^{a}	0.04^{b}	0.06	< 0.001
k	0.22	0.24	0.25	0.04	0.873

Values within a row with different lowercase superscripts differ significantly at $P<0.05$.

6.4 Discussion

6.4.1 Effect of forage species on boli characteristics

Forage species did not affect the fresh weight of ingested boli of cows fed indoors. Comparison of the physical and chemical characteristics of the three forages used in this study indicate that they were significantly different in their physical and chemical characteristics (i.e. fragment size, biomechanical properties and structural fibre concentration). This finding is in agreement with Acosta et al. (2007) who observed no difference in the fresh weight of boli from cows consuming different forage species (ryegrass, tall fescue and white clover). Mean fresh weight of the boli in this experiment was greater than that observed by Acosta et al. (2007) (169 vs. 132 g), but mean body weight of cows in this experiment was also greater. The lack of forage effect, but between-cow variation in bolus wet weight, supports other research that suggest cows will manipulate herbage to an extent which overcomes differences in physical characteristics of forage in order to create a bolus that can be swallowed comfortably and safely. Thus the size of the bolus is determined by the animal (Gill et al., 1966; Prinz and Lucas, 1997).

Although weight of the boli did not differ between species, the herbage and saliva content of the boli did. The saliva content of ryegrass boli was 11 – 18% greater than the saliva content of chicory and lucerne boli, likely reflecting the functions of saliva which are to create cohesion between particles and provide lubrication for swallowing (Hutchings and Lillford, 1988; Prinz and Lucas, 1997). Chicory herbage had the least amount of saliva added during mastication, despite chicory having longer and wider leaves than the other forages. It is possible the low DM and fibre (NDF) concentration of chicory, which required one fifth the energy to macerate than ryegrass, eased the manipulation of chicory herbage into a bolus, thus reducing the requirement for added saliva. The results suggest that the requirement of saliva for bolus formation is associated with the biomechanical properties and DM and fibre concentration of herbage. Research to determine whether this relationship applies in subsequent feeding bouts, and the implications on saliva production during the remainder of digestion and rumen buffering would be valuable.

Dry matter content of the herbage influenced DM intake (DMI) rate (g/min), which is consistent with research using fasted cows fed indoors (Cabrera Estrada et al., 2003). Although cows fed chicory increased their intake rate of fresh forage, they were unable to compensate for the low DM content of the feed and attained a low DMI compared with ryegrass and lucerne, both of which had higher DM content. This agrees with other studies that positively correlated DMI of sheep and cows with the DM content of fresh herbage (John and Ulyatt, 1987; Cabrera Estrada et al., 2003; Acosta et al., 2007). It suggests, that in studies that have observed similar DMI between cows fed diets of ryegrass only or ryegrass and chicory (Muir et al., 2015; Minneé et al., 2017), the cow must ingest a larger number of chicory boli in order to achieve this. This concept is supported by Gregorini et. al. (2013b) who reported increased ingestive mastication for cows grazing chicory relative to those grazing ryegrass in a field study. Those authors noted similar grazing time for both forages, suggesting the rate of swallowing individual boli of chicory was more rapid than for ryegrass. Therefore, the findings of these studies support the conclusion that the DM content of forage influences DMI rate.

6.4.2 Effect of forage species on comminution of herbage

Mastication, as ingestive chewing and rumination, is the main process responsible for reducing feed to fragment sizes that can pass from the rumen. The extent of comminution differed between forages in this study, where mastication reduced 32, 38 and 35% of chicory, lucerne and ryegrass herbage to particles less than the 2 mm threshold thought able to pass from the rumen (Ulyatt et al., 1986). This reduction in particle size is less than the 50% reported from cows consuming fresh red clover (Reid et al., 1962), but greater than the 12-25% observed for fresh legumes and grasses (McLeod and Minson, 1988; Boudon et al., 2006; Acosta et al., 2007). The variation in extent of fragment size reduction may be due to the different hunger states of the animals used in the studies. Nevertheless, many of the studies reported that herbage with a greater DM content and fibre concentration, or greater implied ‘toughness’ tended to be reduced to a smaller particle size compared to ‘weaker’ forages containing less fibre. This is supported by the present study, where ryegrass contained the greatest concentration of fibre and required the greatest energy to macerate,

also had less DM in the large particle fraction compared to chicory. Further investigation of the PSD of the large (> 4 mm) fragments in the boli, showed particles from ryegrass boli had the shortest in median length, and contained a greater proportion of particles between 4 - 40 mm. Whereas the weaker and less fibrous chicory herbage had the greatest proportion of large particles in the > 4 mm fragment pool. In a comparison between green panic (*Panicum maximum* var. trichoglume) and ryegrass herbage, Wilson et al. (1989b) also observed that the more fibrous and tougher green panic was reduced to smaller mean particle size during mastication than the less fibrous ryegrass herbage. Similarly, Lee and Pearce (1984) observed that the greatest reduction in feed particle size occurred in feeds with the greatest fibre content and toughness. These findings indicate that species with greater fibre content or toughness are chewed more extensively to allow bolus formation, resulting in greater fragmentation of herbage material. The relationships between herbage fibre concentration and comminution were, however, not linear supporting observations in section (4.3.3) and the literature section (2.6.1). Therefore, herbage fibre concentration explains some of the variation in particle size reduction, but other characteristics (not determined in this study) must also contribute; the process of mastication is complex, and is likely influenced by multiple feed and animal factors (Acosta et al., 2007).

6.4.3 Effect of forage species on herbage nutrient release

An important function of mastication is cell rupture to allow microbial access, and the release of rapidly fermentable nutrients (soluble carbohydrates and nitrogen). Mean loss of nutrients from fresh chicory, lucerne and ryegrass (Table 6.5) was at the lower end of the range reported for cows consuming forage indoors (30 – 37% and 19 – 26% loss of WSC and N respectively) (Boudon and Peyraud, 2001; Boudon et al., 2006; Acosta et al., 2007). The proportion of WSC released was the same for chicory as it was for ryegrass, despite the lesser fragmentation of chicory herbage in boli compared to ryegrass which suggests that the extent of comminution is not the sole determinant of nutrient release during ingestion. A similar conclusion was made by Acosta et al. (2007) who compared the nutrient release from white clover (*Trifolium repens* L.) with temperate grass species (*Lolium perenne* L. and *Festuca arundinacea* Schreb.) during mastication. There was no correlation

between nutrient release and extent of fragmentation but, nutrient release was associated with the toughness of the herbage.

The greatest amount (g/kg DM) of WSC released in this study, was from chicory herbage which contained the greatest concentration of WSC and was also the weakest herbage of the three forages evaluated, requiring the least energy to macerate. These observations suggest that during mastication, crushing of the weaker chicory herbage as it was manipulated into a bolus, could have ruptured cells and disrupted tissue allowing for WSC to escape without a reduction in fragment size. In contrast, a similar proportion of WSC was released from the tougher, ryegrass herbage despite the greater extent of fragmentation in ryegrass boli. Which supports the conclusion of previous chapters, that the characteristics that influence nutrient release are specific to forage.

Furthermore, the higher proportion of N loss from lucerne herbage might also be explained by its greater N concentration and the very low strength of the lucerne lamina material which is where most plant N is stored (Wilman and Altimimi, 1982). Similarly, Acosta et al. (2007) also showed that forages with greater N concentration, particularly a greater intracellular N (Total N – NDIN) concentration as observed in lucerne herbage, tended to release more N. While the toughness of herbage and concentration of nutrients explains some of the variation in nutrient release, this study indicates that the extent of comminution of herbage is not a main driver for the release of nutrients during mastication.

A greater proportion of WSC than nitrogenous compounds was released during mastication, which is consistent with the findings in sections 4.3.4 and 5.3.2, and other studies (Boudon and Peyraud, 2001; Boudon et al., 2006; Acosta et al., 2007). Boudon and Peyraud (2001) suggested that because most plant N is in chloroplasts and proteins, which are large molecules and typically associated with organelles, they are far less easily released from ruptured cells during ingestive mastication than smaller, soluble carbohydrates which are stored in the cytoplasm or vacuole and can diffuse through pores or across the cell wall of intact cells (Akin, 1986; 1989). However, it is the amount and timing of availability for the rumen microflora that affects utilisation, rather than the proportion

released. The amount of WSC released was 10 times greater than N release from chicory, but only 2 and 3 times greater than N from lucerne and ryegrass, respectively. While the amount of N released was greater from lucerne compared with ryegrass, the rate of release (g/min) was 46% faster from ryegrass. More information on nutrient release, and the factors that affect it, could help explain differences in observed production or N excretion in livestock consuming different diets (Hutton et al., 2011; Totty et al., 2013; Minneé et al., 2017). It has been suggested that the balance of nutrients made available to the rumen affects the efficiency of nutrient use (Cole and Todd, 2008), particularly in intermittent feeding regimens, such as pastoral grazing where herbage is allocated once or twice a day. Improved understanding of availability dynamics in the rumen, may enable improved feeding management to mitigate environmental impacts of pastoral grazing.

6.4.4 Implications for digestion in the rumen

The degradation kinetics of boli material were studied using the *in sacco* method, and the soluble 'A' fraction is a measure of nutrient release by mastication. However, the extent of release by washing *in sacco* bags were substantially less than that washed through cheese cloth using the method of Boudon and Peyraud (2001) and presented in Table 6.6. There are two possible causes for this difference. Firstly, the calculation assumes that the quantity of NDF in the bolus is the same as the quantity of NDF in the feed eaten, which is unlikely as some fibre will be solubilised during ingestion. Burke (2004) demonstrated that the soluble fraction (A) of NDF for chicory, lucerne and ryegrass was 0, 40 and 21%, so while Boudon and Peyraud (2001) did not feel the assumption would introduce a bias, the values for soluble NDF observed by Burke (2004) and Chaves et al. (2006) were greater for ryegrass and lucerne than that assumed by Boudon and Peyraud (2001). Thus these calculated values may overestimate N loss. The second reason for the disparity was that the larger pore size in cheese cloth would have allowed more material to pass through when washing compared to the nylon bags which had a very small pore size of 35 microns. However, the trends for N release and N solubilisation determined by both methods were similar between the three forages evaluated and, thus the *in sacco* degradation results support the conclusion that lucerne herbage releases more N during comminution than chicory and ryegrass. This finding is largely due

to the greater N concentration in lucerne, where most N is stored in weak leaf tissue that is susceptible to rupture (section 6.4.3). Released N is very rapidly hydrolysed in the rumen (Mangan, 1982; Weisbjerg et al., 1998), if this N is not used by the microflora it is excreted. The greater initial release of N from lucerne could result in poor utilisation N by cows, which may in part explain the greater excretion and high concentrations of N in urine of cows fed lucerne compared to cows fed diets without lucerne (Waghorn et al., under review).

In this study, DM degradation rate of masticated ryegrass (k = 0.22%/h) appeared to be more rapid than for lucerne or chicory (Table 6.6) and this value is greater than that for fresh macerated ryegrass observed in section 5.3.4 (0.04 %/h) and reported in the literature (Burke et al., 2000; Chaves et al., 2006). It is difficult to explain this data. One suggested reason is that the lesser proportion of DM in the A fraction of boli compared to macerated material meant that some of the potentially soluble material was degraded in the rumen, which would likely have been very rapid leading to the high k value demonstrated in this study. Or perhaps the greater amount of saliva added to ryegrass boli may have improved the hydration and microbial colonisation of ryegrass boli particles facilitating more rapid degradation. Further investigation would be required to support this.

Total potential degrability (P) of herbage DM and N was least in lucerne compared with chicory and ryegrass. These data support the findings of Burke (2004) (discussed in section 2.6), and are likely associated with the morphology and chemical composition of the herbage, i.e. that lucerne contains fibrous and lignified stem material whereas the other species did not. However, this study focussed on the effects of ingestive mastication on comminution and nutrient release, to what extent the animal compensates for the lesser degrability of lucerne herbage by subsequent ruminative chewing was not evaluated.

Care is required if the intention is to extrapolate these data to cattle grazing outdoors. Rate of DM intake was greater in this study than demonstrated by others investigating lactating dairy cows grazing perennial ryegrass and white clover (mean 60 vs. 40 g DM/min) (Marotti, 2004; Rutter et al., 2004). The disparity is likely associated with the hunger state of the animals used, as they had

been fasted overnight, and because they were fed indoors cut fresh herbage which would have facilitated higher intake rates. Boudon et al. (2006) compared the ingestive behaviour and its effect on perennial ryegrass herbage that was fed indoors or grazed by cattle and reported that cattle fed indoors had 128% greater DMI rate, heavier boli with less saliva added per gram of DM, larger particle size in the boli and reduced loss of nitrogen during ingestive mastication when compared with the cattle grazing. Therefore, it is likely that greater release of N during ingestive mastication would occur from these forages when grazed due to the additional damage to herbage tissue from the forces applied during prehension and biting of herbage.

6.5 Conclusion

Evaluation of swallowed boli showed that the extent of comminution of herbage and nutrient release after ingestive mastication differed between chicory, lucerne and ryegrass. Boli from cows ingesting chicory contained more herbage with larger particles and less saliva than boli from cows ingesting ryegrass. The proportion of nutrient release was not solely related to the extent of comminution of the feed, but rather the toughness of the herbage and the amount of nutrient. Soluble carbohydrate is more readily released from the feed than nitrogen, and variation in the amount of released nutrient exist between forages. This may go some way to improve our understanding of how forages are utilised.

7

Simulating ingestive behaviour, N metabolism and N excretion of cows grazing swards fertilised with increasing rates of nitrogen

7.1 Introduction

The efficiency of nitrogen (N) utilisation in dairy cows is low. That is, typically only 15 – 30 % of ingested N is captured in product (i.e. body tissue or milk) and the remainder excreted as waste, mostly in urine (Astigarraga et al., 1994; Delagarde et al., 1997; Astigarraga et al., 2002). Nitrogen that is excreted in urine, and on to pasture, is a source of environmental pollution because the concentration of N in a urine patch often exceeds plant capacity for uptake (up to 1000 kg N/ha (Di and Cameron, 2007)). Then, that urinary N is largely converted to nitrate in the soil, which is highly susceptible to leaching (section 2.2). Several studies have demonstrated that N excreted in urine is associated with N intake of livestock (Kebreab et al., 2001; Tas et al., 2006), which is a function of the N concentration in herbage and the amount of herbage eaten (Waghorn et al., 2007). As demonstrated in section 5.4.2, reducing N fertilisation rate decreases herbage N concentration but has little effect on soluble carbohydrate and structural fibre concentrations in non-legume species. It is for this reason that reducing the rate of N fertiliser applied to forage swards has been proposed as a means for improving N use efficiency (NUE) and reducing urinary N excretion from grazing ruminants (Hoekstra et al., 2007a; Dijkstra et al., 2011).

Conversely, reducing N fertiliser applied to swards can decrease herbage growth rate and sward height (section 5.4.1, (Belesky et al., 2000; Binnie et al., 2001; Martin et al., 2017). Furthermore, in some forages (e.g. ryegrass), reducing N fertiliser increases the toughness of herbage (section 5.4.1). These are important considerations for farmers as reductions in sward DM production can influence stock carrying capacity, and a reduction in sward canopy height and increases in herbage toughness can alter livestock grazing behaviour and intake (Ungar and Noy-Meir, 1988; Laca et al.,

1992). Thus, an optimal fertiliser rate for forage species is sought that balances herbage productivity with livestock N use efficiency.

Key elements required to measure the NUE of livestock fed different forages, such as feed intake and ingestive behaviour, production, and N excretion, are difficult and expensive to measure (Gregorini et al., 2013a). Therefore, the use of mechanistic models to predict these processes is a valuable exercise, and a critical first exploratory approach to assess the potential merits of different forages. The purpose of this study was to use the 'MINDY' dairy cow model to explore outcomes of increasing fertiliser N applied to swards of four common forage species on N metabolism and partitioning, and the ingestive behaviour of grazing dairy cows. It was hypothesised that the optimal N fertiliser rate to reduce urine N excretion and maintain or improve milk production from dairy cows would vary among forages. The aim of the exercise was to explore how varying N fertiliser rates applied to swards of chicory, lucerne, plantain and ryegrass, would be predicted to effect dairy cow production and N excretion.

7.2 Materials and Methods

7.2.1 Model

The MINDY (Gregorini et al., 2013a) model was used to simulate effects of feeding a dairy cow diets of four different forages, each fertilised at five different rates of N fertiliser, on ingestive behaviour, N metabolism and excretion. MINDY is a deterministic, mechanistic and dynamic model of a dairy cow, representing diurnal patterns of ingestion, digestion and metabolism, excretion and production. The model is based on explicit relationships among direct (digestion and metabolism) and indirect (feeding environment) influencers of motivation to feed (Gregorini et al., 2013a; Gregorini et al., 2015). MINDY is based on a cluster of six models: 1)'Molly' the dairy cow digestion, metabolism and production model developed by Baldwin (1995) and modified by Gregorini et al. (2015); 2) diurnal fluctuations in the feeding motivation and feed intake 3) sward canopy structure (height, density, distribution of DM and chemical composition in the sward), and herbage quality (including diurnal fluctuation); 4) grazing behaviour; 5) dietary preference and

herbage selection; and 6) foraging bioenergetics. For further detail of the model, the reader is referred to Gregorini et al. (2013a) and (2015).

7.2.2 Model inputs

Forage physical and chemical composition data that were used to create the diets for MINDY are detailed in Table 7.1. The data were obtained from samples of herbage collected in April 2015 from a small plot trial, where four species were grown under 6 fertiliser N rates in a randomised split-plot design with three replicates (full trial design is detailed in section 3.2.1). The species selected for this simulation exercise were: ryegrass, lucerne, chicory, and plantain; and N application rates were: 0, 100, 200, 350 and 500 kg N/ha/y (hereafter termed as 0N, 100N etc.). Methods for herbage dry matter (DM) yield and concentration are detailed in section 3.2.4.

Because MINDY can account for the physical characteristics of forages, ease of breakdown (EOB) of the herbage during ingestion was determined from measured mean punch force of herbage (detailed in section 3.3.1). The calculation assumed that that the EOB of ryegrass herbage fertilised at 200N = 1.0, and the EOB of other herbages were calculated relative to the 200N ryegrass. For example, the mean punch force of 200N ryegrass herbage = 0.96 Newtons/mm^2, thus an EOB of 1 = 0.96 Newtons/mm^2, whereas 0N ryegrass had a mean punch force of 1.14 Newtons/mm^2, therefore converting this by a factor of 0.96 gives an EOB of 1.2.

Laboratory analyses for determination of herbage chemical composition are detailed in section 4.2.3. Values for herbage fat content were only measured in the 200N herbage samples (method 2003.05; AOAC, 2006), and were assumed to be unaffected by fertiliser N application. As discussed in section 4.3.1, the water soluble carbohydrate (WSC) assay underrepresents the concentration of fermentable carbohydrates, so non-structural carbohydrate (NSC) concentration was calculated by the Equation 4.1 (section 4.3.1).

Table 7.1 Chemical composition, nutritive and physical characteristics of herbage from the four species and five rates of fertiliser N application (kg N/ha/y) used in the simulations.

Species	N rate	DM[1] (%)	CP[2]	Fat	NSC[3]	NDF[4]	Ash	CPS[5]	RUP[6]	NPN[7]	EOB[8]	Extended leaf height (cm)	Herbage mass (kg DM/ha)
			---------- (g/g DM) ----------					------- (Proportion of CP) -----					
Chicory	0	12.4	0.136	0.039	0.238	0.207	0.240	0.270	0.400	0.001	0.8	22.3	2060
Chicory	100	11.4	0.144	0.039	0.238	0.216	0.230	0.278	0.351	0.001	0.8	33.3	2670
Chicory	200	11.3	0.167	0.039	0.228	0.215	0.220	0.283	0.348	0.001	0.7	35.7	3300
Chicory	350	10.2	0.198	0.039	0.209	0.232	0.188	0.306	0.330	0.002	0.7	39.4	3830
Chicory	500	9.5	0.209	0.039	0.211	0.239	0.167	0.379	0.312	0.002	0.6	47.9	4060
Lucerne	0	17.7	0.270	0.045	0.095	0.477	0.118	0.401	0.28	0.002	0.6	37.0	3070
Lucerne	100	17.3	0.302	0.045	0.096	0.439	0.116	0.417	0.291	0.003	0.6	34.5	3040
Lucerne	200	17.5	0.307	0.045	0.098	0.430	0.118	0.436	0.282	0.003	0.6	33.4	3105
Lucerne	350	17.2	0.295	0.045	0.091	0.452	0.116	0.451	0.274	0.003	0.6	35.8	3126
Lucerne	500	17.9	0.284	0.045	0.099	0.451	0.120	0.452	0.279	0.005	0.6	34.7	3115
Plantain	0	18.2	0.147	0.042	0.211	0.393	0.164	0.104	0.493	0.007	1.0	21.5	2140
Plantain	100	17.4	0.151	0.042	0.222	0.380	0.161	0.109	0.468	0.007	1.0	33.7	2410
Plantain	200	16.7	0.153	0.042	0.224	0.377	0.160	0.250	0.490	0.009	0.9	36.4	2885
Plantain	350	15.8	0.170	0.042	0.231	0.361	0.153	0.255	0.487	0.009	0.8	39.4	3135
Plantain	500	15.5	0.180	0.042	0.238	0.346	0.150	0.320	0.484	0.010	0.8	42.9	3640
P. ryegrass	0	23.9	0.171	0.045	0.157	0.484	0.108	0.333	0.320	0.001	1.2	20.0	1790
P. ryegrass	100	20.1	0.183	0.045	0.154	0.470	0.112	0.370	0.315	0.015	1.0	23.0	2300
P. ryegrass	200	19.4	0.184	0.045	0.159	0.461	0.116	0.368	0.316	0.015	1.0	25.3	2580
P. ryegrass	350	17.8	0.220	0.045	0.138	0.448	0.111	0.390	0.305	0.021	0.9	30.3	2710
P. ryegrass	500	17.6	0.220	0.045	0.138	0.447	0.113	0.390	0.300	0.032	0.9	35.2	3210

[1] DM, dry matter; [2] CP, crude protein; [3] NSC, non-structural carbohydrate; [4] NDF, neutral detergent fibre, [5] CPS, soluble crude protein; [6] RUP, rumen undegradable protein; [7] NPN, non-protein nitrogen; [8] EOB, ease of breakdown.

The particulars of one cow used in the study detailed in Chapter 6, were used to initialise MINDY, and are detailed in Table 7.2.

Table 7.2 Values used to initialise MINDY

Parameter	Input
Breed	Friesian
D.O.B.	27 Jul 2010
Live-weight	423 kg
BCS	4.5
Mating date	23 Oct 2012
Calving date	01 Aug 2013

7.2.3 Simulation methods

Each of the four forages, were simulated separately to explore the effect of N fertiliser rate on the ingestive behaviour, N metabolism and excretion within species. Each simulation spanned 20 days, from 01 – 20 March 2014, with the last 5 d comprising the 'measurement' period. The simulation was set in Hamilton, New Zealand. Within each scenario, five clones of MINDY (referred to as a 'cow' hereafter) were offered one of the five dietary N fertiliser treatments. Cows were offered a 24-hour break of pasture as determined by the allocated treatment, with the grazing area defined to provide sufficient area to provide a 30 kg DM/cow/d allowance based on herbage mass (Table 7.1). Cows grazed within a simulated herd of 200 cows that were milked twice daily, at 0600 and 1600 h.

The outputs requested from MINDY were: total daily dry matter intake (DMI, kg/cow), N intake (kg/cow), milk yield (L/cow), milk-solids yield (kg/cow), N excreted in milk, urine and faeces (g/cow), N retained or removed from body tissue (g/cow) and urine volume (L/cow); average daily urinary urea concentration (g/L), rumen ammonia concentration (mol/L), jaw movements/bolus, bolus weight (g), swallowing frequency (bolus/min) and herbage intake rate (g DM/min); and mean distribution of small, medium and large particles in swallowed boli.

7.3 Results

7.3.1 Simulated effect of N fertilisation rate on dairy cow ingestive behaviour

Predicted daily dry matter intake (DMI) increased with increasing rate of N fertiliser applied when grazing swards of chicory, plantain, and ryegrass, but not when grazing lucerne (Figure 7.1a). Compared to the 0N swards, increasing fertiliser to 500N increased DMI by 5.0, 5.2 and 2.7 kg DM/cow/d on chicory, plantain, and ryegrass diets respectively (20 – 43% increase). Correspondingly, predicted N intake increased in non-legume species with increasing fertiliser N applied and ranged from 281 – 836 g N/cow/day across all diets (Figure 7.1b).

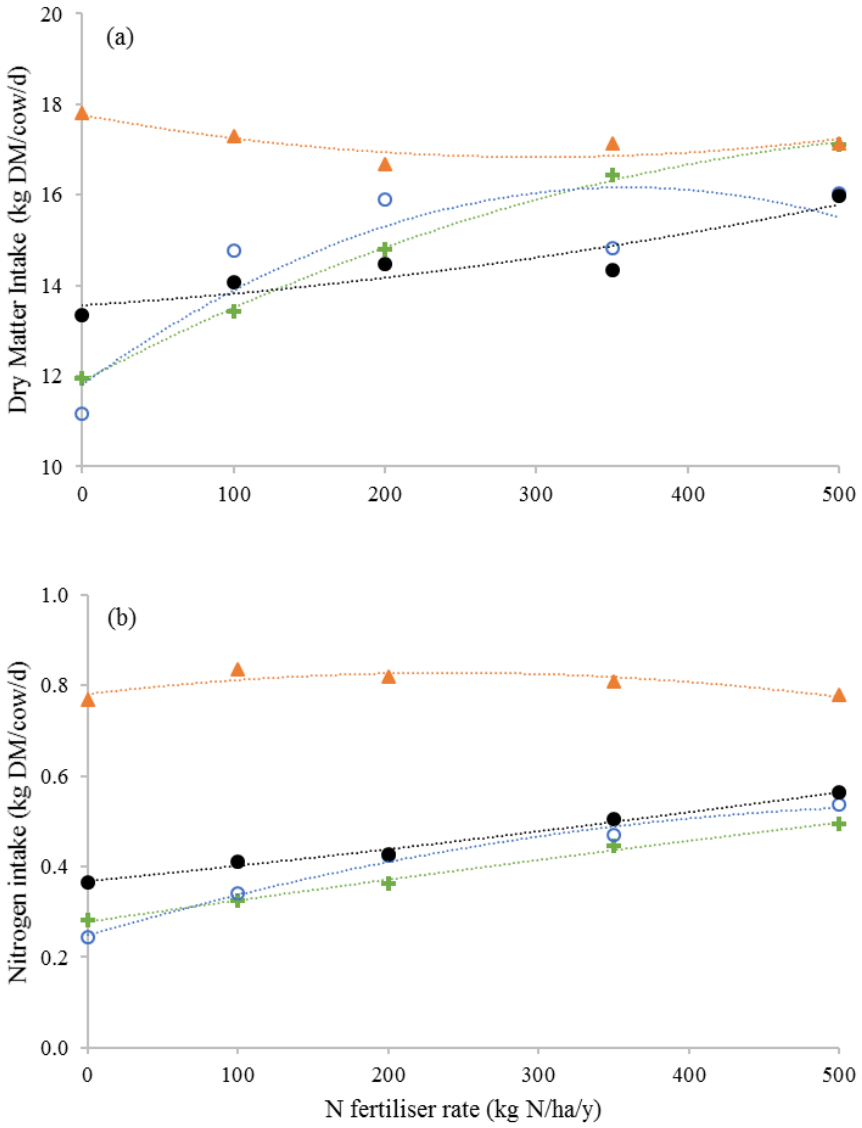


Figure 7.1 Predicted dry matter (a) and nitrogen (b) intake of dairy cows grazing swards of chicory (○), lucerne (▲), plantain (+) and perennial ryegrass (●) fertilised with increasing rates of nitrogen.

Herbage intake rate (g DM/min) was predicted to increase with increasing fertiliser N application in chicory, plantain and ryegrass diets (Table 7.3). The greatest increase in intake rate was predicted when cows were offered chicory, increasing by 35.5 g DM/min when N rate increased from 0 – 500N, followed by ryegrass (19.1 g DM/min increase) and plantain (13.6 g DM/min increase). With ryegrass and plantain, increased DMI rate appeared to be associated with the swallowing of fewer but larger boli as N rate applied increased, whereas in chicory the model predicted that a greater number of boli were ingested per minute at the higher rates of fertiliser N application. The predicted number of jaw movements required to form a bolus was largely unaffected by N fertiliser rate in lucerne, chicory and plantain, but ryegrass resulted in more jaw movements per bolus at greater rates of fertiliser N (350 – 500N) compared with the lower application rates (7.6 vs. 4.0 movements/bolus).

Table 7.3 Predicted daily mean and standard deviation of the number of jaw movements per bolus, bolus weight (g), bolus swallowing frequency (number of boli per minute) and herbage intake rate of cows fed herbage of four forage species fertilised at five rates of fertiliser N.

	N fertiliser rate (kg N/ha/y)					
	0	100	200	350	500	SEM
Jaw movements/bolus						
Chicory	4.1 ± 0.6	5.0 ± 0.9	4.4 ± 0.7	4.9 ± 0.7	4.6 ± 0.8	0.17
Lucerne	8.4 ± 1.6	8.5 ± 1.5	8.2 ± 1.5	8.7 ± 1.5	8.0 ± 1.6	0.13
Plantain	7.7 ± 1.4	8.5 ± 1.5	8.5 ± 1.4	8.3 ± 1.1	8.8 ± 0.9	0.18
P. ryegrass	4.5 ± 0.7	3.5 ± 0.5	3.9 ± 0.9	7.2 ± 1.4	7.9 ± 1.6	0.88
Bolus weight (g)						
Chicory	127.8 ± 10.3	126.9 ± 21.9	129.5 ± 11.1	128.8 ± 11.5	128.2 ± 12.0	0.43
Lucerne	43.8 ± 15.3	44.3 ± 11.4	44.1 ± 14.0	47.5 ± 14.5	40.1 ± 12.8	1.17
Plantain	43.6 ± 13.0	55.1 ± 13.1	67.2 ± 13.0	86.9 ± 15.6	96.5 ± 20.2	9.77
P. ryegrass	6.6 ± 0.4	7.6 ± 3.7	12.3 ± 3.0	39.5 ± 13.9	48.8 ± 12.7	8.82
Swallowing frequency (bolus/min)						
Chicory	1.6 ± 0.2	2.1 ± 0.4	2.3 ± 0.4	2.4 ± 0.5	2.9 ± 0.5	0.20
Lucerne	3.3 ± 0.6	3.2 ± 0.4	3.2 ± 0.5	3.1 ± 0.4	3.4 ± 0.5	0.04
Plantain	3.1 ± 0.5	2.7 ± 0.3	2.5 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	0.16
P. ryegrass	7.2 ± 0.7	9.0 ± 1.6	7.7 ± 2.3	3.4 ± 0.7	3.0 ± 0.5	1.20
Herbage intake rate (g DM/min)						
Chicory	23.7 ± 10.5	38.8 ± 6.8	43.2 ± 7.6	48.8 ± 8.1	59.2 ± 9.7	5.86
Lucerne	33.1 ± 9.1	33.3 ± 4.9	33.0 ± 5.8	34.8 ± 6.0	32.4 ± 5.9	0.40
Plantain	32.9 ± 5.3	35.9 ± 5.3	38.5 ± 6.0	42.9 ± 6.8	46.5 ± 8.3	2.44
P. ryegrass	16.1 ± 1.5	18.2 ± 3.2	18.7 ± 6.3	31.4 ± 5.8	35.2 ± 5.6	3.91

The predicted particle size distribution (PSD) of feed in swallowed boli is illustrated in Figure 7.2. The PSD of chicory and lucerne boli appears unaffected by N fertiliser rate. However, the proportion of large particles in the boli of plantain and ryegrass tended to increase with increasing N fertiliser rate. The greatest increase was observed in the ryegrass swards where ryegrass fertilised at 500N had 5 times the amount (on a DW basis) of large particles compared with 0N ryegrass. Correspondingly, the proportion of small particles declined at the higher rates of fertiliser N application.

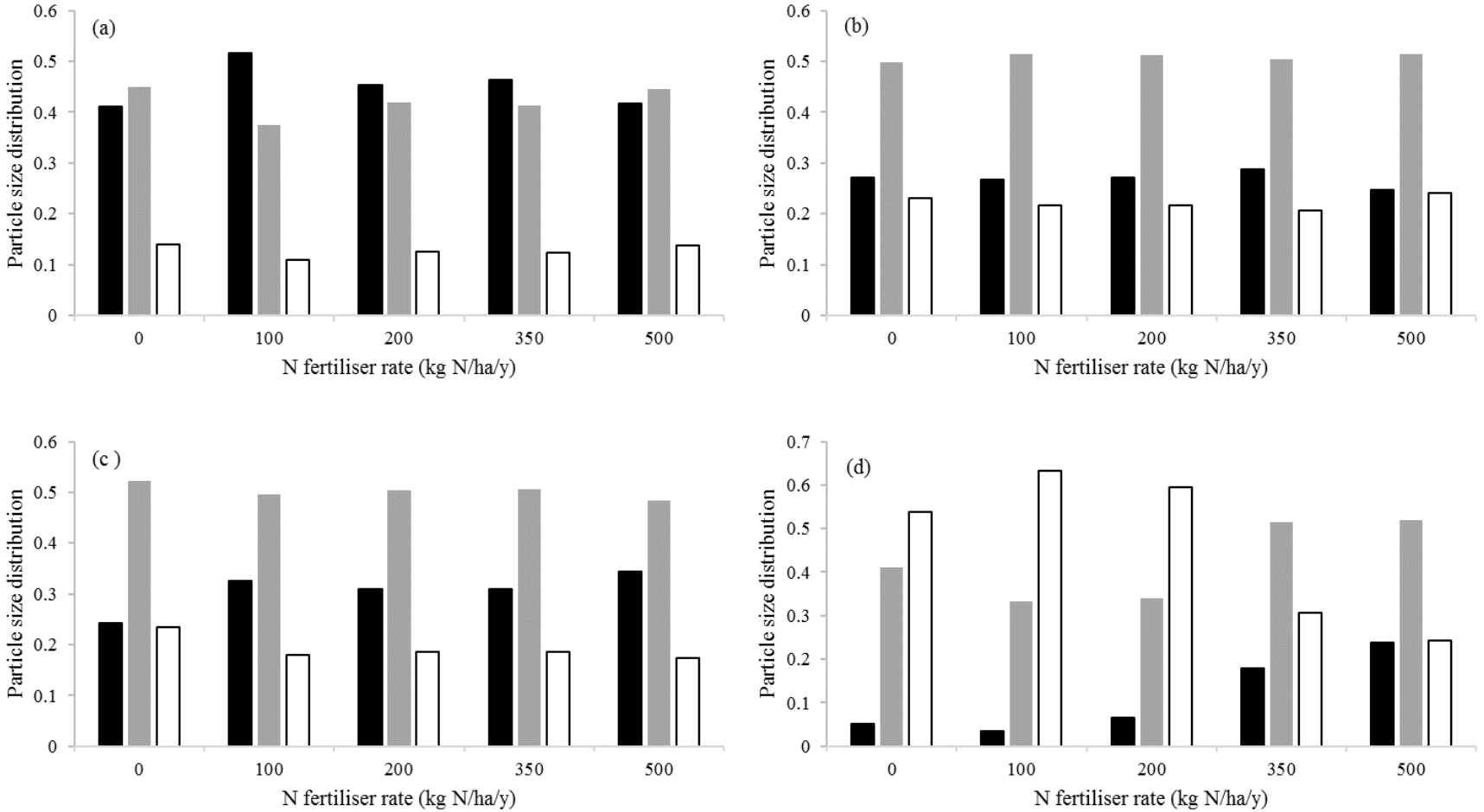


Figure 7.2 Predicted proportional distribution of large (>4.8 mm; ■), medium (1.2 – 4.8 mm; ■) and small (< 1.2 mm; □) particles of herbage in swallowed boli from dairy cows fed chicory (a), lucerne (b), plantain (c) and perennial ryegrass (d) herbage fertilised at five rates of N fertiliser.

7.3.2 Simulated effect of N fertilisation on N metabolism and partitioning in the dairy cow

The NSC: CP ratios increased when fertiliser N was applied at rates exceeding 200N in chicory and ryegrass, and there was a slight increase in the ratio in plantain herbage fertilised at 500N compared with unfertilised plantain (Table 7.4).

Table 7.4 Non-structural carbohydrate: crude protein ratio of herbage from four species fertilised at increasing rates of nitrogen.

	N fertiliser rate (kg N/ha/y)				
	0	100	200	350	500
Chicory	0.6	0.6	0.7	0.9	1.0
Lucerne	2.8	3.1	3.1	3.3	2.9
Plantain	0.7	0.7	0.7	0.7	0.8
P. ryegrass	1.1	1.2	1.2	1.6	1.6

The model predicted mean rumen ammonia concentration to increase by 15, 7, 4 and 10 mmol/L in chicory, lucerne, plantain and ryegrass herbage respectively when N fertiliser application increased from 0 – 500N (Figure 7.3 a). The largest increases (~ 162%) were predicted in chicory, and diets where fertiliser application exceeded 200N. Predicted plasma urea N concentrations followed the same pattern as rumen ammonia concentration (Figure 7.3 b).

The predicted partitioning of dietary N by cows fed forage fertilised at increased rates of N is summarised in Figures 7.4 a-d. Nitrogen partitioned to milk was predicted to decline by an average of 23% when chicory, plantain, and ryegrass fertilised with increasing rates of N were fed. Nitrogen partitioned to faeces also tended to decline with increasing fertiliser N when chicory or ryegrass were grazed. While N partitioned to urine increased by 35% for chicory diets, and by 14 and 29% for plantain and ryegrass diets respectively when fertiliser applied increased from 0 – 500 kg N/ha/y. In contrast, the partitioning of N in cows grazing lucerne was largely unaffected by N fertiliser application rate.

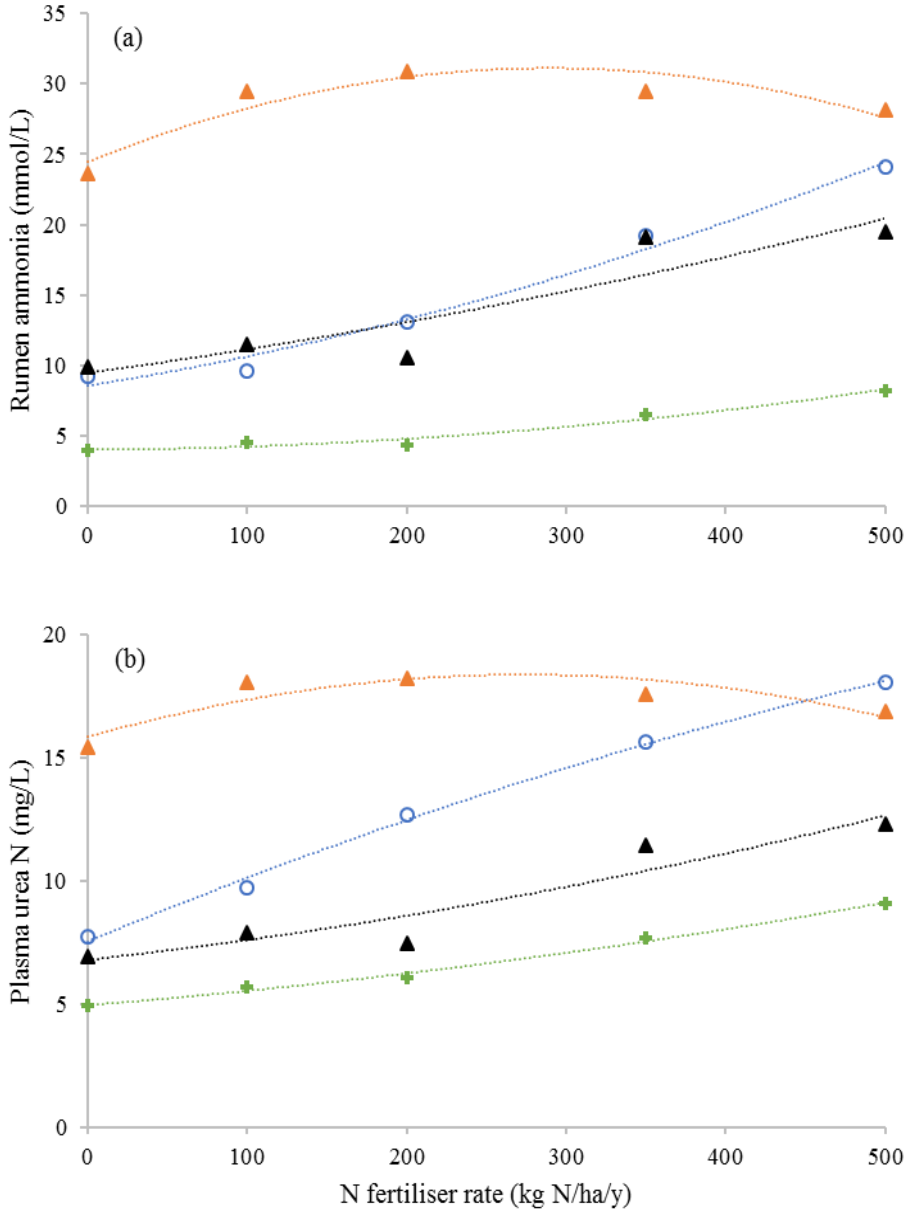


Figure 7.3 Predicted daily mean rumen ammonia (a) and plasma urea nitrogen (b) concentrations of dairy cows fed diets of chicory (○), lucerne (▲), plantain (+) and perennial ryegrass (●) fertilised with increasing rates of nitrogen.

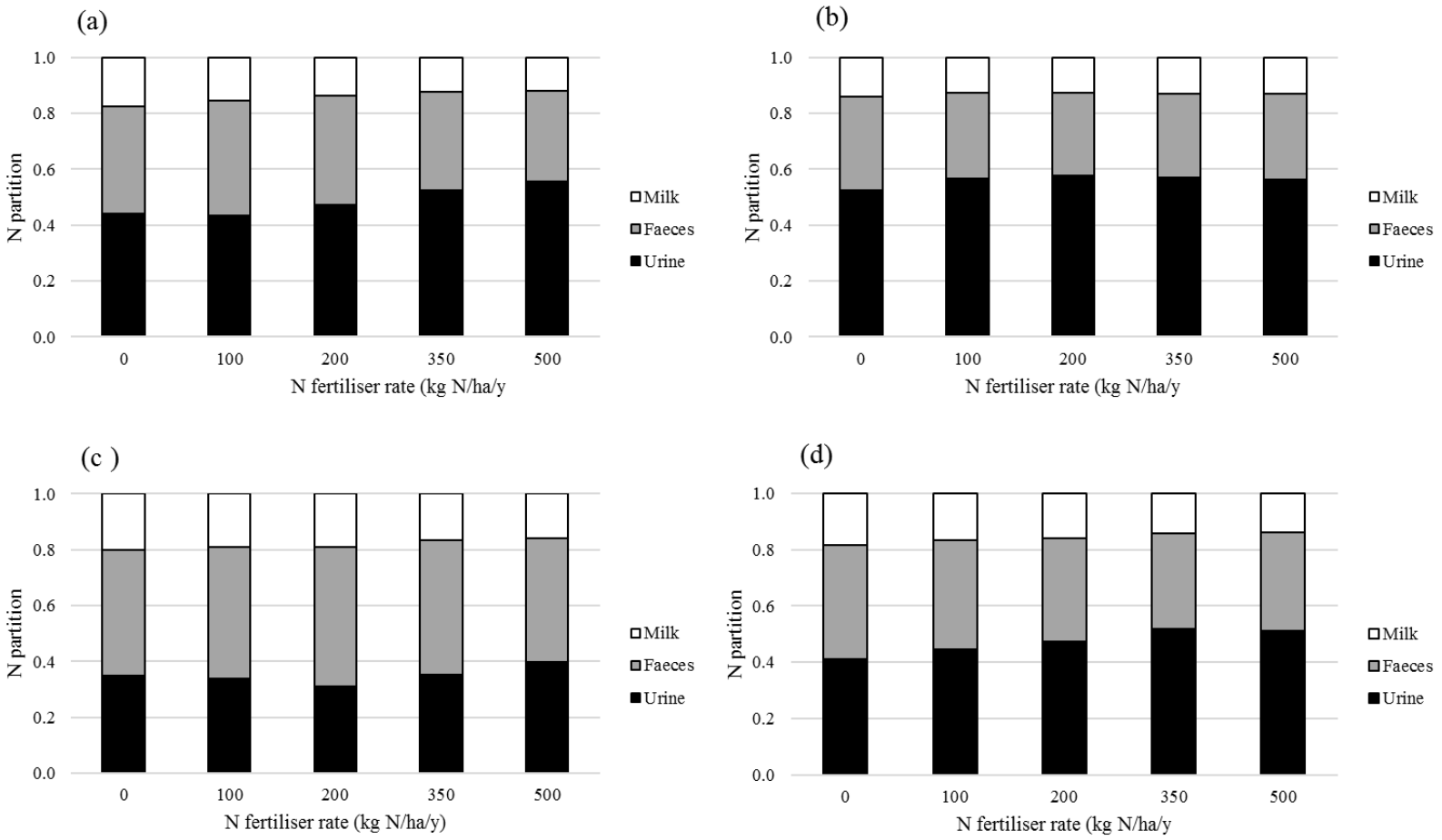


Figure 7.4 Predicted partitioning of nitrogen (N) to urine (■), faeces (■), and milk (□) in dairy cows fed chicory (a), lucerne (b), plantain (c), and ryegrass (d) fertilised with increasing rates of fertiliser N (0 – 500 kg N/ha/y).

When MINDY grazed plantain and ryegrass diets, predicted urinary N concentration and total daily N excretion increased when fertiliser N application rates exceeded 200N. Whereas in chicory diets, N excretion increased progressively with N application rate, but urine N concentration peaked when fed lucerne diets receiving 200N (Figure. 7.5 a-d). Predicted N excretion in urine from MINDY when fed lucerne did not appear to be altered by N fertiliser rate.

The model predicted a positive linear association between N intake and rumen ammonia concentration and urine N excretion ($R^2 > 0.85$; Figure 7.6 a, b). Simulations within species suggest a quadratic association between N intake and rumen ammonia concentration when plantain or ryegrass diets were fed, with the point of infection occurring when N intake exceeded 0.4 kg/cow/day (Figure 7.6 a). Strong linear responses were observed between N intake and daily urinary N excretion for all species ($R^2 > 0.9$; Figure 7.6 b).

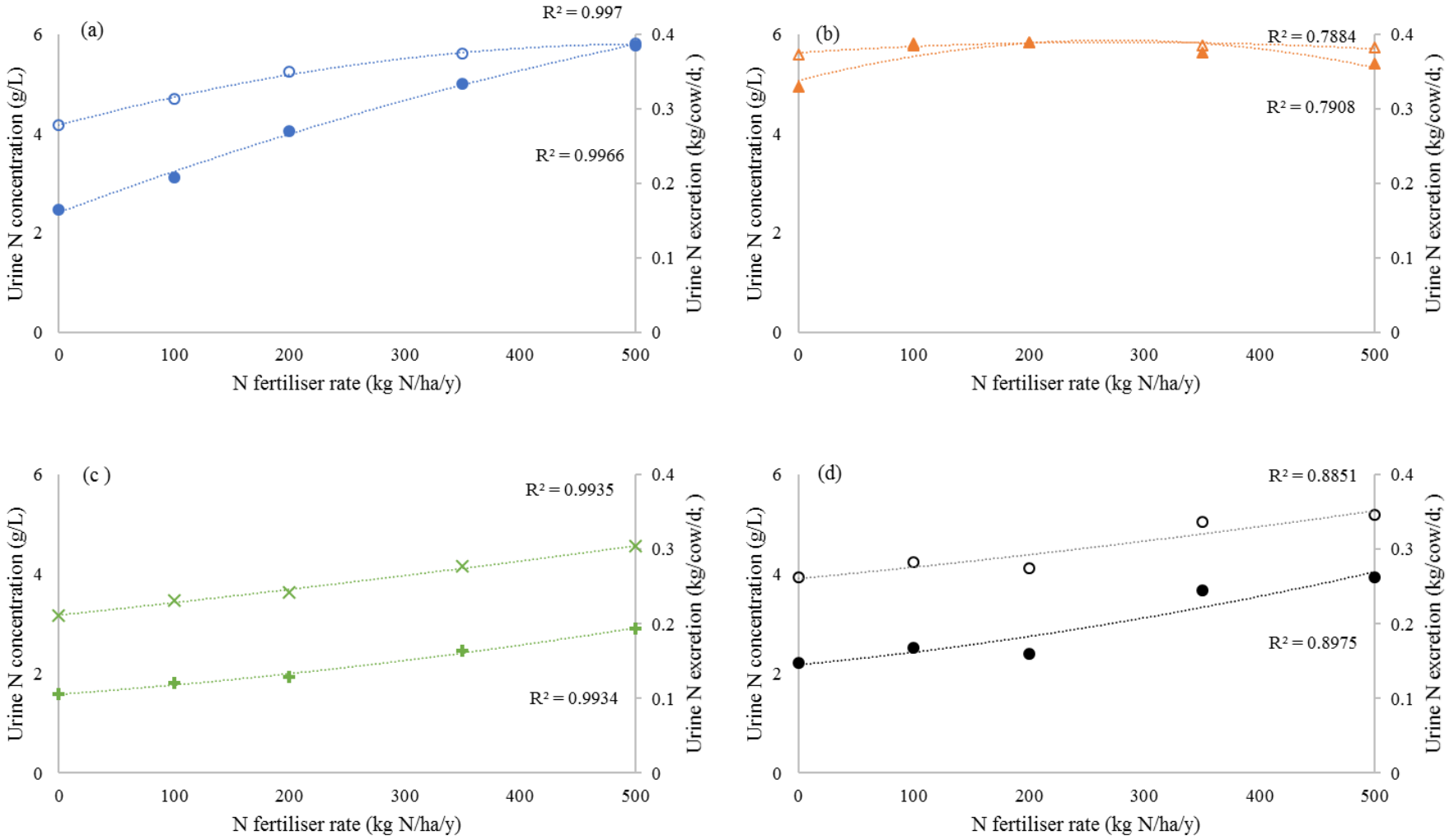


Figure 7.5 The predicted effect of increasing N fertiliser rate applied to swards of chicory(a), lucerne (b), plantain (c), and perennial ryegrass (d) on the concentration of urea in urine (open symbols) and total daily urinary N excretion (closed symbols).

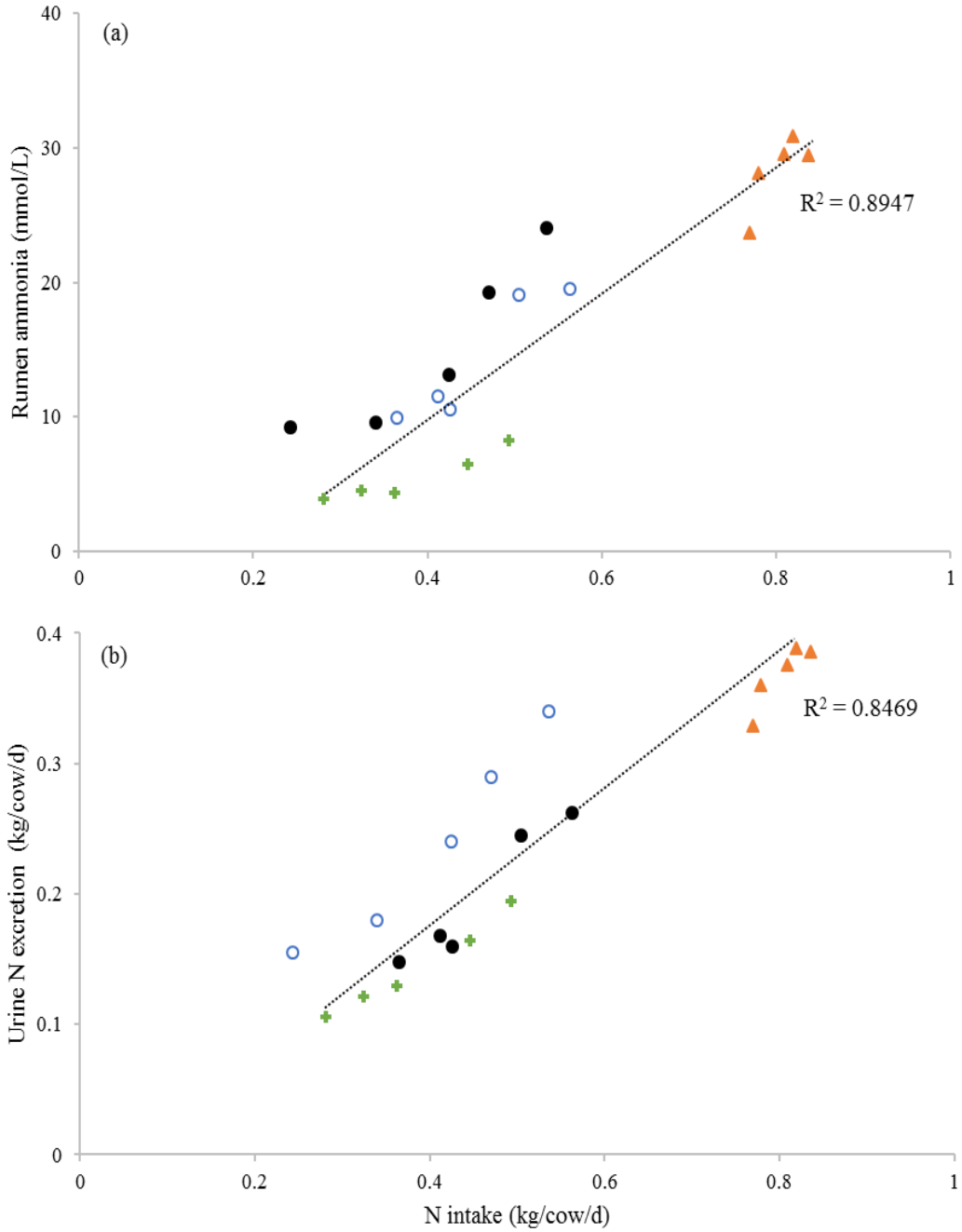


Figure 7.6 Simulated relationship between N intake and rumen ammonia (a) concentration, and daily urinary N excretion (b) of dairy cows fed chicory (○), lucerne (▲), plantain (+) and perennial ryegrass (●).

Predicted milk (L/cow/d) increased with increasing N fertiliser application, for cows grazing chicory (12.3 – 15.3 L/cow/d), plantain (12.3 – 14.8 L/cow/d), and ryegrass (12.4 – 13.4 L/cow/d) (Figure 7.7 a). Similarly, milk-solids (MS) yield (kg/cow/d) also increased with increasing N rate, by 22, 17 and 8% from diets of chicory, plantain, and ryegrass, respectively (Figure 7.7 b). Unlike plantain and ryegrass where the milk production response was linear, chicory demonstrated a diminishing response when fertiliser application rate exceeded 200N (Figure 7.7). Predicted Milk and MS yield declined marginally (1%) when N fertiliser was applied to lucerne swards. The predicted efficiency of milk solids production (g MS/g N eaten), declined with increasing N fertiliser application rate by an average of 35% in diets of chicory, lucerne, and plantain (Figure 7.7 c).

Investigation of the relationships between intake and MS yield across forages showed that N intake was strongly positively correlated with MS yield (Figure 7.8 b), and a moderate correlation between MS and DMI (Figure 7.8 a). Dry matter intake was also moderately positively associated with CP concentration of herbage ($R^2 = 0.71$; $y = 0.0185x + 0.8481$)

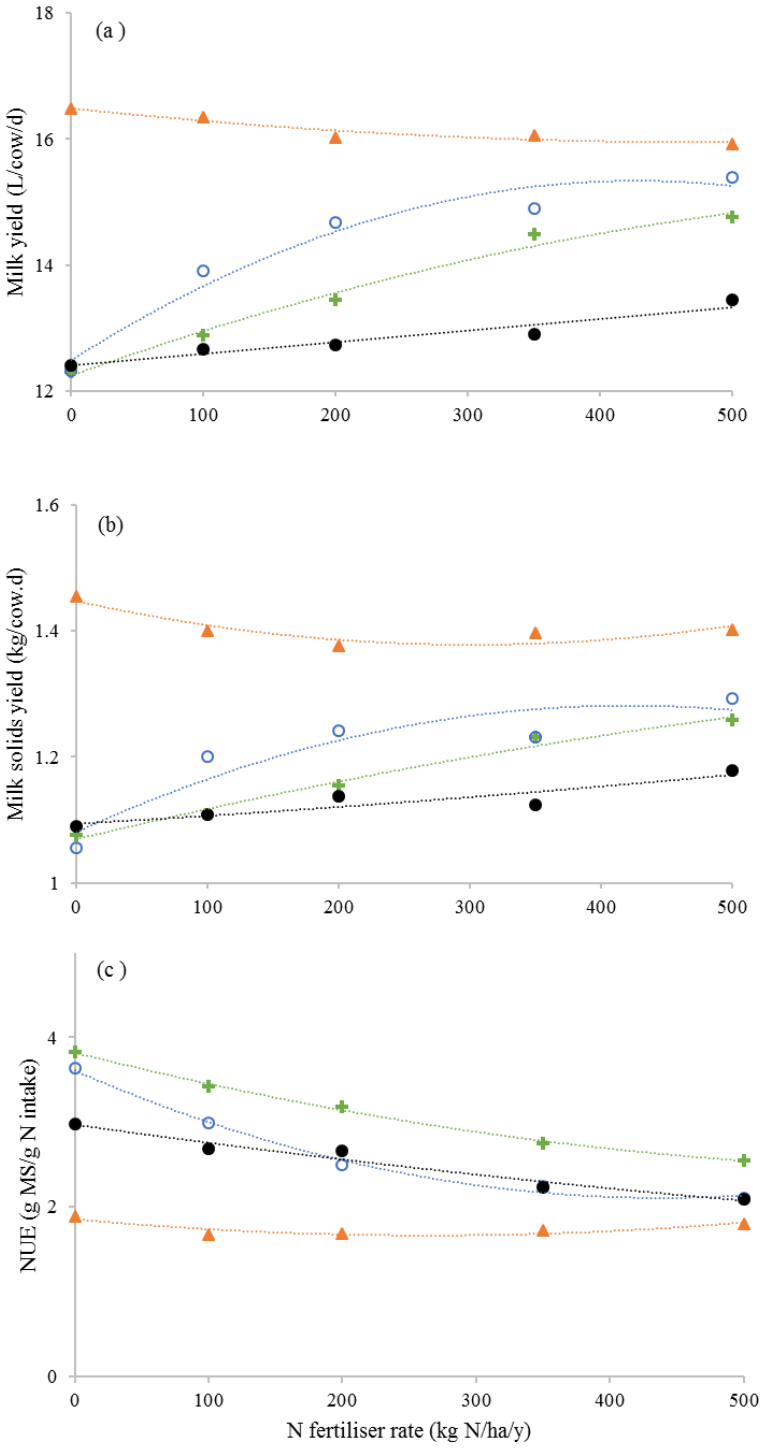


Figure 7.7 The predicted effect of increasing N fertiliser rate applied to swards of chicory (○), lucerne (▲), plantain (+) and perennial ryegrass (●) on milk yield (a), milk solids yield (b) and (c) the nutrient use efficiency (NUE) of dairy cows.

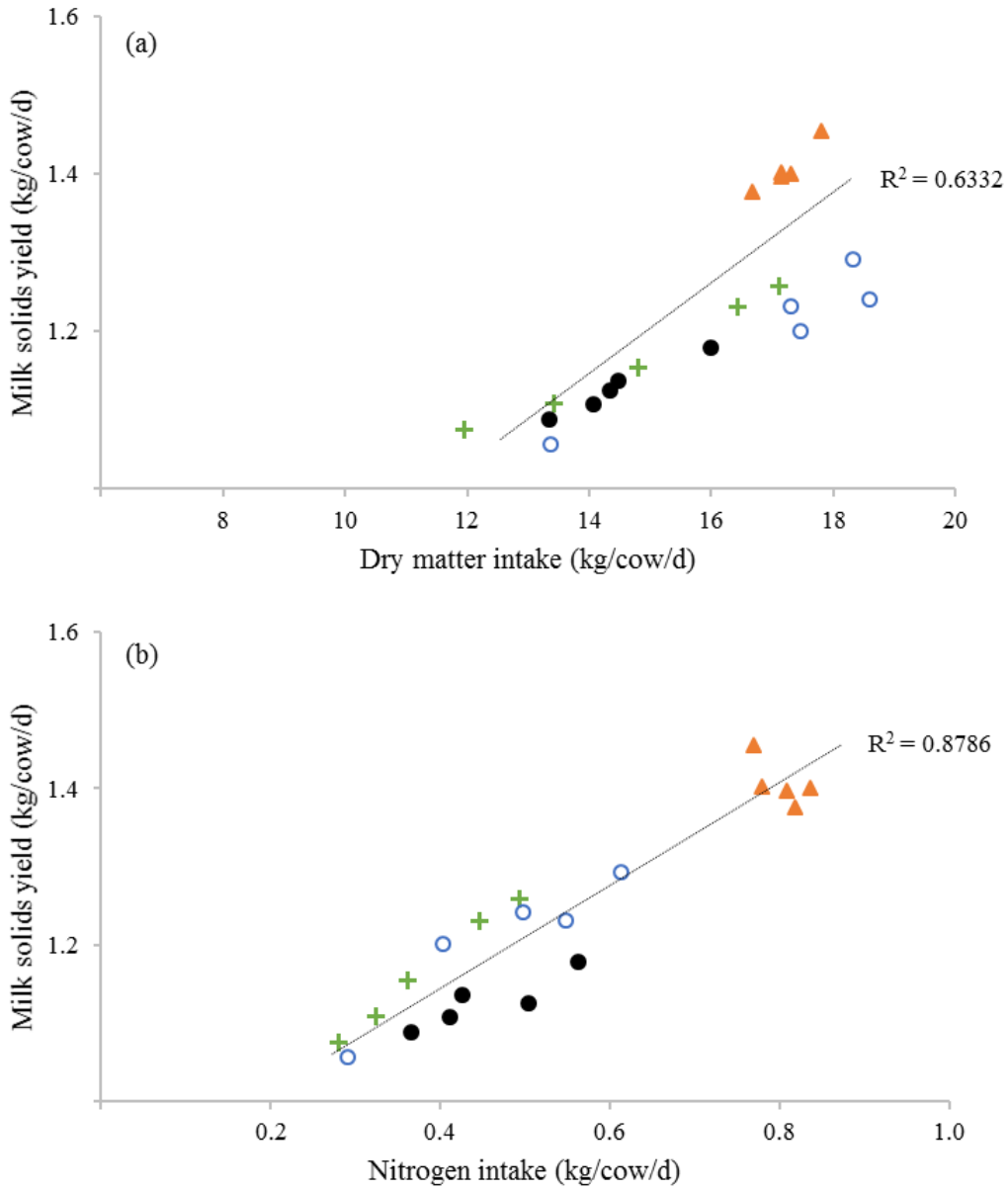


Figure 7.8 The predicted relationship between milk production and dry matter (a) and nitrogen intake (b) of dairy cows grazing swards of chicory (○), lucerne (▲), plantain (+) and perennial ryegrass (●).

7.4 Discussion

The multiple herbage-based variables used as inputs into the model (i.e. sward physical characteristics and herbage chemistry) make it difficult to elucidate the precise drivers for influencing DMI in this study. However, the simulations undertaken did make it possible to explore the practical aspects of cows ingestive behaviour, N metabolism and production, when grazing forages grown under different N fertiliser rates.

Overall, the model predicted DMI and MS production within the range observed in studies from cows grazing similar diets under similar N fertiliser regimes, at the same stage of lactation (Woodward et al., 2010; Minneé et al., 2012; Muir et al., 2014). It appears the model, however may be under-predicting bolus weight, particularly for ryegrass as measurements detailed in Chapter 6 and other studies involving cows fed ryegrass or lucerne, reported much greater weights (range of 88 – 173 g FW/bolus) than predicted in this simulation (Gill et al., 1966; Boudon and Peyraud, 2001; Boudon et al., 2006; Acosta et al., 2007). Though it must be recognised that the study in Chapter 6, and most others noted, were conducted in a tie-stall, feeding cut fresh herbage, where according to the study of Boudon et al. (2006) boli from grazing cows would be expected to be smaller than cattle fed indoors.

7.4.1 Intake and feeding behaviour

The MINDY model simulates the ingestive behaviour and digestive processes of a grazing dairy cow in response to both external (i.e. sward height) and internal (i.e. rumen ammonia concentration) stimuli (Gregorini et al., 2013a). In agreement with the literature (Delagarde et al., 1997; Valk et al., 2000; Hodgson et al., 2009), dry matter intake (DMI) of cows was greater when grazing swards fertilised at greater rates of N applied (> 350N) compared to those grazing swards grown under low N fertiliser (< 200N). Predicted intakes of highly fertilised ryegrass swards may have been in response to increased sward height and mass and reduced herbage strength (i.e. ease of breakdown) associated with increasing N application (Table 7.1, and section 5.3.1). Physical changes in the highly fertilised swards would be expected to increase accessibility and ease of prehension of herbage during grazing (Ungar and Noy-Meir, 1988; Laca et al., 1992; Penning et al., 1994; Rook et al., 1994), resulting in larger boli size and greater intake rate predicted by the model (Table 7.3). A similar trend was evident during simulation of cows grazing plantain diets, where applying N fertiliser to swards was predicted to increase DMI by 5 kg/cow/d (43% increase) likely in response to the measured increase in sward height (+ 21 cm) and mass (+ 1500 kg DM/ha) as documented in section 5.3.1. With chicory, measured sward height also increased with N application (Table 7.1 and section 5.3.1), and predicted DMI similarly increased with increasing N fertiliser rate up to 200N with associated effects on bolus characteristics (Table 7.3). The decline in predicted DMI of cows grazing chicory grown under high N fertiliser application rate (> 200N), and lucerne, however could be associated with high predicted rumen ammonia concentrations (> 16 mmol/L;

Figure 7.3 a) and plasma urea concentrations (Figure 7.3b) which would reduce incentive to continue grazing (Gregorini et al., 2013), and is consistent with the literature concerning ruminants (Conrad et al., 1977; Cosgrove et al., 1999). High rumen ammonia concentrations were less commonly predicted (range 3.9 – 19.5 mmol/L; diurnal variation data not reported here), and predicted plasma urea concentrations were lower, when cows grazed ryegrass and plantain diets. Lower rumen ammonia values would have been due to lesser total daily N intake of cows fed plantain and ryegrass compared with chicory and lucerne diets, as well as lower soluble CP (CPS) and higher rumen undegradable protein (RUP) in plantain herbage compared to the other diets (Table 7.1) (Huntington and Archibeque, 2000). This simulation study suggests that DMI of dairy cows can be indirectly altered by the N fertiliser application rates, and the effects are forage specific.

Model predictions of particle size distribution (PSD) of ingested boli varied between forages, as did the predicted in response to increasing N fertiliser application rate for each forage (Figure 7.2). Generally ingested boli from cows grazing the broader leaved dicot forages (chicory, lucerne and plantain) contained a greater proportion of large particles (> 4.8 mm) than predicted for ryegrass (a monocot) boli (average 0.40 vs. 0.15, respectively). Predicted boli PSD data compliments measurements detailed in Chapter 6, that showed that ryegrass herbage was reduced to a greater extent in boli than chicory and lucerne herbage. When increasing fertiliser N was applied to the swards, the predicted effect on PSD of herbage in lucerne and chicory boli was minor. In plantain and ryegrass boli, however, the proportion of large particles increased with increasing N application. The increase was smaller in plantain boli (0.10 increase) but more marked in ryegrass boli (from a proportion of 0.05 in the boli at 0N to 0.24 at 500N) Correspondingly the proportion of small particles in ryegrass and plantain boli declined when fertiliser N increased from 0 – 500N. The changes in boli PSD, especially in ryegrass, were probably affected through the influence of N on the chemical composition and physical presentation of the swards (i.e. longer leaves, greater sward mass, reduced herbage strength and DM content). While there are no known livestock based studies to confirm these trends, the predictions agree with findings in Chapter 5 where ryegrass herbage grown under high fertiliser N was weaker, and fragmented to a lesser extent by mechanical maceration in the laboratory, than tougher ryegrass herbage grown under low N fertiliser rates.

The rate and extent of particle size reduction is important because of effects on rumen fill, clearance, and intake (Allen, 1996; Kammes and Allen, 2012). The greater proportion of small particles in the low fertilised ryegrass and plantain boli may enable more rapid digestion and passage (Poppi et al., 1981a; Poppi et al., 1981b) leading to greater DMI. But model predictions did not support higher intakes of diets with greater reduction in particle size. Passage of particles from the rumen is known to be a complicated and dynamic process, influenced by several factors (Kammes and Allen, 2012) including feed intake rate. It is likely that the slower predicted feed intake rates of cows grazing the unfertilised swards (0N; Table 7.3), may be due to difficulty in harvesting (shorter, tougher swards), as well as reduced degradation rate (Chapter 5) all influenced model predictions of lesser DMI of cows grazing unfertilised compared with fertilised swards.

7.4.2 Nitrogen excretion

Predicted total urinary N excretion from cows increased by 90 – 220 g/cow/d as more fertiliser N was applied to the non-legume forages (0 – 500N) (equating to up to a 134% increase; Figure 7.5 a-d). The largest increases tended to occur in N application rates exceeding 350N in plantain and ryegrass diets, whereas predicted urine N excretion increased linearly with increasing N application from cows grazing chicory ($R^2 = 0.99$). The increase in N excretion was likely a function of N intake (Figure 7.6 a, b) and due to differences in N partitioning (Figure 7.4 a-d). Strong linear or exponential responses of increased N excretion with N intake have been well documented (Castillo et al., 2000; Kebreab et al., 2001; Kebreab et al., 2002; Spek et al., 2013). Particularly, in studies feeding forage-based diets due to the high content of soluble and rumen degradable protein, leading to elevated rumen ammonia concentrations (Huntington and Archibeque, 2000), which were also predicted by the model (Table 7.5). Predicted rumen ammonia concentrations were within the range observed in studies of dairy cows under intermittent feeding (Rodriguez et al., 1997). When calculated as daily means, predicted rumen ammonia increased with increasing N fertiliser application in agreement with published studies involving cows fed ryegrass (Peyraud et al., 1997; Astigarraga et al., 2002). The simulations conducted in this study illustrate the relationship between increasing dietary N intake and N excretion and indicate the importance of fertiliser N management to manipulate urinary N.

Plantain diets were predicted to result in lower rumen ammonia concentrations and concomitantly, lesser N excretion than the other forages modelled in this study. Simulations predicted a higher proportion of dietary N would be partitioned to faeces when cows were fed plantain (0.47) compared to the other forages evaluated (mean 0.32). This will be a consequence of the relatively low N concentration in plantain herbage with greater proportion of rumen undegradable N, relative to the other forages. This may also in part explain the decrease in urine N excretion and concentration observed in studies where plantain comprised part of the diet of dairy cows (Totty et al., 2013; Box et al., 2016; Minneé et al., 2017). These findings present opportunity to manipulate N partitioning through inclusion of plantain in the diet of dairy cows.

7.4.3 Milk production and NUE

Predicted milk yield and MS production were largely within the range reported from studies investigating the milk production of dairy cows consuming similar diets and levels of intake (Woodward et al., 2010; Minneé et al., 2012; Muir et al., 2014). Milk-solids production increased when cows grazed non-legume swards with increasing rate of fertiliser N applied (an increase of 0.09 – 0.24 kg/cow/d, or 8 – 22%). While the predicted increase in MS from cows grazing plantain was positively related to N fertiliser applied to swards, the MS response from cows grazing chicory and ryegrass was marginal at fertiliser rates exceeding 200N. This reduced response at higher rates of N fertiliser, agrees with studies by Valk et al (2000) and Astigarraga et al. (2002). Extensive reviews by Kebreab et al. (2001) and Castillo et al. (2001a) concluded that the effect of increasing CP concentration of the diet on milk production is negligible when the diet contains a high proportion of RDP N and where N intake was sufficient to meet cow requirements for lactation and maintenance (NRC, 2001), as was observed in the current study. The model simulations in this study support the conclusion of those reviews, that there are no benefits of increasing N fertiliser application rate beyond 200N to ryegrass and chicory for MS production, but suggest that greater rates than 200N could be applied to plantain swards to improve MS production.

Although predicted MS production was strongly positively correlated with DMI and N intake (Figures 7.8 a-b), the efficiency with which the N is used declined by an average of 35%, and the proportion of dietary N partitioned to milk declined by 30% in non-legume diets under increasing N fertiliser

applications. There was also a slight negative effect of N fertiliser to lucerne on MS production (Figure 7.7 b), indicating that applying fertiliser to lucerne is unwise. It would be valuable to investigate how N fertiliser application to mixed swards containing lucerne influences affects whole sward NUE.

7.5 Conclusions

Model predictions provided a likely outcome from the fertiliser treatments on both dairy cow production and digestive metabolism of N. These results suggest that adjusting to the application rate of N fertiliser to chicory, plantain and ryegrass provides a means to manipulate animal NUE and reduce N losses to the environment. In the context of this study, predictions of MS production and N excretion by the MINDY model suggests application of N fertiliser at 200 kg/ha could be the optimal, for compromise between MS production and total daily N excretion for chicory, plantain, and ryegrass swards. At this rate, predicted DMI and MS production from cows grazing chicory was maximised and no further gains were achieved through additional N fertiliser. Predictions for plantain and ryegrass showed higher (>200N) applications of N resulted in small increases in MS production, but the amount of N excreted increased exponentially. The simulations confirmed negative production and environmental consequences from applying N fertiliser to lucerne swards.

8

General Discussion

8.1 Introduction

In New Zealand, grazed forages provide the majority of the diet of dairy cows. Forages provide New Zealand with a relatively low cost feed system for production of dairy products, and a competitive advantage in the global dairy market (Dillon et al., 2005). The efficiency of the system is maximised by high levels of consumption of pasture herbage. The dominant species grown is perennial ryegrass (Holmes et al., 2007) because this species can produce large amounts of herbage (up to 20 t/DM/ha/y), is tolerant of a wide range of environments and management practices, and is responsive to N fertiliser to promote plant growth (Kemp et al., 2000a). The adoption of N fertilisers to New Zealand farming in the 1990's, and improvement in grazing management have increased herbage productivity, and contributed to the intensification of dairying in this country (through increased stock numbers and stocking rates). Over a similar period, however, N loading into land has increased (Scarsbrook and Melland, 2015) and the quality of New Zealand's fresh water resources has declined (Verburg et al., 2010; Ballantine and Davies-Colley, 2014), and it is the N loss from agricultural systems that has been identified as a main contributor for environmental N pollution.

Herbage from forage species generally contains greater concentration of CP than is required by the animal, and in ryegrass, the addition of N fertiliser can exacerbate this (Tamminga, 1992a; Kolver et al., 1998; Whitehead, 2000). Excess dietary N is largely excreted, in the urine, and is a potential source of N pollution to groundwater (leaching) and nitrous oxide emissions to the atmosphere (Whitehead, 1995; Johnson et al., 2005). Forage species differ in their herbage CP concentration, and several reviews have shown a strong positive association between N intake and N excreted in the urine (Castillo et al., 2000; Kebreab et al., 2001; Parsons et al., 2011; Moorby, 2014). However, in some studies, measured differences in N excretion or urinary N concentration have not wholly been explained by N intake (Box et al., 2016; Minneé et al., 2017). The theory of synchronising nutrient supply, i.e. fermentable carbohydrates (i.e. non-structural carbohydrates, NSC) and N, suggests that maximal efficiency of N use for microbial protein synthesis (MPS) will occur when these nutrients are balanced (~ 5:1 ratio of

NSC to N) (Tamminga, 1992a). Parsons et al. (2011) and Moorby (2014) also demonstrated that urine N excretion decreases as the ratio of NSC: N decreases. Generally, in forages, the amount of N is too high and NSC content is too low, and the imbalance results in the high urinary N excretion of cows consuming diets of forage species compared to those fed concentrate diets (i.e. as is common in the United States of American and Canada) (Rearte, 2005; Belanche et al., 2013). Beever and Cottrill (1994) suggested that the low utilisation of N and high N loss to urine observed in cows fed forage based diets is related to the pattern with which N and energy (from degradation of CHO) are made available to the rumen microflora. Meaning, that as well as the total concentration of N and NSC in herbage being unbalanced in quantity, the timing that N and degraded CHO is available to the microbes during digestion may also be unbalanced. Because much of the herbage N is contained within the plant cells, it is only accessible to microbial degradation once cell rupture (through mastication) has occurred. Therefore, the extent and timing of cell rupture will influence the delivery of N to the rumen, which in turn has potential to effect utilisation of N.

While there have been comprehensive studies investigating the ruminal degradation of N and DM from herbage of forage species (Waghorn et al., 1989; Burke et al., 2000; Chaves, 2003; Burke, 2004; Chaves et al., 2006), less is known about the extent of degradation and nutrient release during the initial phase of feed degradation, that is ingestion. A wide range (22 - 86%) of nutrient release from legume and grass species has been reported (Bryant, 1964; Boudon and Peyraud, 2001; Boudon et al., 2006; Acosta et al., 2007), but the characteristics of the herbage that influenced this were not determined. As a result, this study sought to expand upon previous work by developing methods to quantify and compare the nutrient release from a range of common forage species, and to determine the key characteristics driving variation. It was anticipated that by better understanding nutrient release from herbage during initial maceration we would improve our understanding of why some forage species result in reduced loss of N to urine when fed to cows, compared with that from other forage diets. Furthermore, determining whether N release and the ratio of N:NSC can be manipulated through management practices, such as varying N fertiliser applied to swards, could be used to inform fertiliser management practices to achieve balance between herbage production and loss of N to the environment.

8.2 Between-species variation in nutrient release during comminution

Comparisons between the forages evaluated by maceration (Chapter 4), in association with N fertiliser application rate (Chapter 5), and ingestive mastication by dairy cows (Chapter 6) showed that the amount of CP released during comminution (by mechanical maceration or ingestive mastication) differs widely between forage species (0.2 – 13.7 g/100 g of DM). This finding agrees with previous studies (Burke, 2004; Acosta et al., 2007) and enables the acceptance of the null hypothesis that nutrient release differs between forages (section 1.2). In general, the least amount of CP released (g/100 g DM) during comminution was from plantain herbage and the greatest from lucerne (equating up to a 15-fold variation in release). Investigation into the characteristics of herbage that effect CP release during maceration, in the forage comparison study, revealed that the characteristics influencing CP release also differ between forages. There was no single common characteristic able to explain CP or fermentable CHO (measured as water-soluble carbohydrate, WSC) release across all forages. While moisture content of herbage was positively associated with CP and WSC release across forages, comparisons within forages showed a negative relationship in some forages, and positive in others. Contrary associations, like this one, have been reported elsewhere in the literature (Bryant, 1964; Lees et al., 1981; Boudon and Peyraud, 2001; Acosta et al., 2007), and attempts to determine an equation to attribute combinations of characters influencing CP release using multi-variate analysis were unsuccessful. It is suggested that the characteristics of the forages selected for this study, being a range of dicot and monocot species, were too dissimilar to elucidate the main drivers across species and future studies might consider comparing more similar forages. Therefore, under the context of the present study we cannot fully accept our second hypothesis that relationships exist between certain herbage characteristics and nutrient release. Further, those characteristics that influence nutrient release from one species cannot be confidently extrapolated to another.

Maceration of the five forages (Chapter 4), however, did indicate that species that contained the greatest concentrations of CP and NPN also tended to release the most CP during mechanical maceration. These data were combined data from the N fertiliser study (Chapter 5) and a curvilinear relationship was established between the proportion of CP release and herbage CP ($R^2 = 0.67$; Figure 8.1) and note the relationship is strengthened.

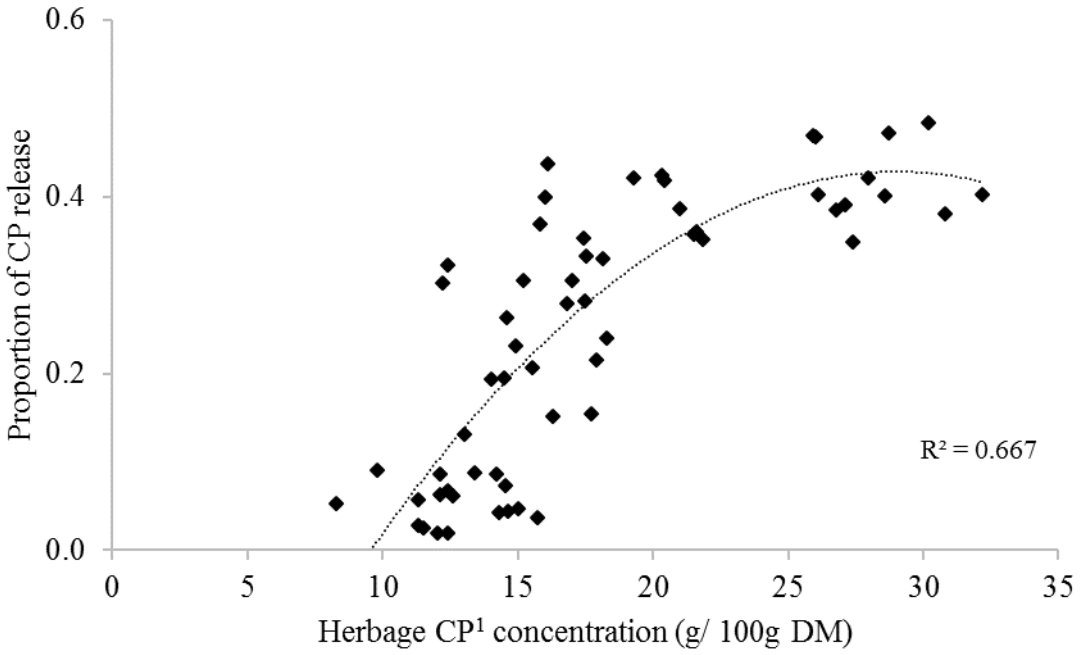


Figure 8.1 The correlation (R2) between the proportion of crude protein (CP) released and herbage CP concentration (g/ 100g DM) from five species. Equation: $y = -0.0011x^2 + 0.0656x - 0.5238$

Release of WSCs also varied between species (range 6.2 – 20.8 g/100 g DM), but was more extensive than observed for N, where at least 85% of WSC were released during mechanical maceration, irrespective of forage species. As with N release, no single common herbage characteristic was strongly correlated with WSC release (section 4.3.5), in part because of the high release of WSC from all forages. Within forages, however, some relationships were observed. For example, WSC release was moderately positively associated with WSC concentration in lucerne, plantain and ryegrass herbage (R^2 0.47 – 0.65, Table 4.7). Herbage toughness of ryegrass, and leaf strength of lucerne and plantain was positively associated with WSC release, suggesting that, at least for these species, the tougher or stronger the herbage is, the more cells will rupture during maceration resulting in greater release of WSC.

The observation in that WSC is more extensively released than CP (Chapters 4 and 5), supports findings reported elsewhere (Boudon and Peyraud, 2001; Boudon et al., 2006; Acosta et al., 2007). Those authors concluded that the relative difference in release was associated with the relative size of WSC and N molecules. Water-structural carbohydrates tend to be smaller molecules compared to large protein molecules, such as those associated with photosynthesis. The disparity in release of CP vs. WSC

suggests that cell rupture during comminution is insufficient to allow all CP to escape. Furthermore, the immediately released CP is likely to be mainly comprised of the smaller and readily fermentable NPN molecules which would have implications on the rate of ruminal ammonia production and thus potentially NUE (Trevaskis, 2003; Hall and Huntington, 2008).

Tamminga et al. (1994) suggested a ratio of available CHO to N of ~ 5:1 for optimal microbial protein synthesis. Comparing the amount of WSC released with CP released from herbage of the five forages in this study showed that the release of CP exceeded WSC release from lucerne, but release from ryegrass and clover were below the ratio, whereas that from chicory is nearer to the optima and plantain was well above (ratio 17:1). These results may explain why the predicted (Chapter 7) rumen ammonia concentrations of dairy cows grazing lucerne and ryegrass were greater than that from cows consuming ryegrass, and similar observations from experiments feeding cows diets of lucerne and ryegrass despite the difference in total CP concentration of the diets being small (Waghorn et al, in press). Similarly, observations of very low rumen ammonia concentration and urine N concentration (Minneé et al., 2017) of cows fed plantain may also be explained by the findings of this study that demonstrated a low quantity of N released from plantain herbage compared to other forage, and the high ratio of NSC: N release.

Species differ in the amount of CP and WSC that is released from herbage during comminution. The quantity of nutrient released, the variation in the ratio of CP and WSC released, and the forms of N released (protein vs. NPN) will affect the efficiency of microbial protein synthesis, and thus NUE. Knowledge of nutrient release can aid understanding of why differences in NUE occur between forage that have similar concentration of CP in herbage.

8.3 The potential to improve nitrogen use efficiency and reduce N excretion

In the forage comparison study (Chapter 4) it was determined that nutrient release varies between forages, and in some release was influenced by certain characteristics of the herbage. It is accepted that forage grass species accumulate N beyond what is required for growth (Whitehead, 2000), but it is not known whether other forage species accumulate N. The association between herbage CP concentration and CP release determined in the forage comparison study presented an opportunity to explore whether

varying N fertiliser applied to forage species would alter CP release during comminution of herbage. Knowledge of the extent to which this could be manipulated through N fertiliser application rate and could help inform fertiliser management regimes to minimise urinary N excretion. To address this opportunity, measurements were undertaken of herbage characteristics, nutrient release, and degradation of herbage from four species (chicory, lucerne, plantain and ryegrass) grown under a range of N fertiliser applications (0 – 350 kg N/ha/y) (Chapter 5). The study confirmed reports in the literature, demonstrating that the concentration of CP and NPN increases in herbage of non-legumes with increasing rate of fertiliser N applied, (Whitehead, 2000; Martin et al., 2017). Herbage CP concentration continued to accumulate in ryegrass, chicory and plantain as fertiliser N application increased, beyond the point where the requirement for growth or accumulation of mass had ceased or declined (Tables 5.2 and 5.3; Figure 5.2. 2 a-b).

The amount of CP released with maceration was also associated with herbage CP and NPN concentration in the fertiliser rate study (Chapter 5) further supporting the findings of the species comparison study (Chapter 4). The effect of CP concentration on CP release tended to be more prevalent in autumn where linear responses observed in ryegrass, chicory and plantain. In spring, however, the effect was only observed in ryegrass. These results suggest the third hypothesis, that N fertiliser rate applied to swards can influence release of nutrients, can only be partially accepted, as release was influenced by N application in all non-legumes in autumn, but only ryegrass in spring. The disparity between seasonal responses may be associated with herbage growth, because in spring the chicory and plantain were continuing to accumulate mass at all N fertiliser rates and there was less 'luxury' accumulation of N in cells. In contrast growth rates were declining when fertiliser application exceeded 200 kg N/ha/y in chicory, plantain and ryegrass, in autumn, and in ryegrass in spring, which could have led to accumulation of N and the increase in the amount of CP released. Therefore, particular care ought to be taken when applying N fertiliser in autumn because of the greater potential to accumulate excess N, and the amount of N released has implications for NUE and N excretion. If reducing the amount of immediately solubilised (i.e. released from cells) CP reduces N loss to urine, these data suggest a maximum application rate of 200 kg N/ha/y to avoid excess loss from cows fed chicory, plantain and ryegrass in autumn.

Chapter 5 demonstrated a marked effect of varying N fertiliser application on the physical presentation and chemical composition of non-legume swards, particularly ryegrass. The greater herbage mass of more highly fertilised ryegrass has been shown to promote greater DMI and feeding rate of cows when compared to cows grazing swards receiving less N fertiliser (Delagarde et al., 1997). Therefore, it was postulated that increasing rates of fertiliser N to other forage species would also increase DMI of cows, but that there would also be increased partitioning of dietary N to urine. Simulation modelling (Chapter 7), using sward chemical and physical data gathered in the N fertiliser study (Chapter 5) was used to explore implications of feeding diets of herbage grown under increasing rates of fertiliser N application (0 – 500 kg N/ha/y) to dairy cows. The greater herbage mass, larger leaves, weaker herbage tissue of the more highly fertilised plantain and ryegrass swards was predicted to increase daily DMI and intake rates of dairy cows. Whereas predicted DMI of cows grazing chicory would be improved at fertiliser rates of 200 compared to 0 kg N/ha/y, predicted DMI declined when rates exceeded 200 kg N/ha/y. Similarly, predicted DMI decreased for cows grazing fertilised compared to unfertilised swards of lucerne. The decreases in DMI predicted in heavily fertilised chicory or fertilised lucerne swards may have been associated with high soluble CP (CPS) content of herbage, which would have led to the greater ruminal ammonia concentrations. In the model, this increased rumen ammonia would likely have reduced incentive for grazing and thus DMI (Gregorini et al., 2013a). Overall, the predicted MS production of cows was associated with DMI (R^2 = 0.63) and N intake (R^2 = 0.88), and although the additional DM eaten by cows grazing plantain swards fertilised at 500 compared to 200 kg N/ha/y increased MS production, the response of cows grazing chicory and ryegrass fertilised at rates exceeding 200 kg N/ha/y was marginal.

Predicted urinary N excretion increased with increasing fertiliser N application rate applied to swards, in line with expectations based on herbage CP concentration (and NSC: CP ratios) (Chapter 5). Greatest predictions of urinary N excretion and urinary N concentration was from cows grazing lucerne diets, and least from cows grazing plantain diets. While urinary N excretion was predicted to increase linearly from cows fed diets of chicory fertilised with increasing rates of N (0 – 500 kg N/ha/y); total daily N excreted in urine was predicted to only increase markedly when rates of N fertiliser was exceeded 200 kg N/ha/y. The increase was associated with increases in dietary N intake, and greater partitioning of N to urine from forage grown under higher fertiliser rates.

The low predicted rumen ammonia concentrations and urinary N excretion of cows grazing plantain also support predictions of NUE in Chapters 4 and 5, and supports existing literature (Box et al., 2016; Bryant et al., 2017; Minneé et al., 2017) that feeding dairy cows diets containing plantain will reduce urinary N excretion.

In terms of managing swards to reduced N excretion, the findings from this research suggest that an application rate of 200 kg N/ha/y to ryegrass swards is optimal for balancing N excretion with herbage production and MS production. Similarly, herbage DMI was predicted to peak at c. 200 kg N/ha/y in autumn, suggesting at this time of year any fertiliser N exceeding rates equivalent to 200 kg N/ha/y would not improve livestock performance. Plantain herbage had NSC: CP ratios closest to the optima at all fertiliser N rates and the lowest N excretion. Considering swards of plantain continued to accumulate mass when fertiliser was applied above 200 kg N/ha/y and milk-solids production was not predicted to decline, then N application exceeding 200 kg N/ha/y could be justified, without excessive loss of N to the environment.

Applying N fertiliser to lucerne swards is not recommended, as this does not improve herbage DM yield, but increases herbage soluble N content, and would likely result in increased urinary N excretion.

8.4 Future research

- None of the herbage characteristics evaluated in this study explained why the release of N from plantain was considerably lower than that from other species evaluated. Assessment of the biomechanical properties of plantain suggest it is relatively tough yet it was fragmented to the greatest extent during mechanical maceration. This may suggest plantain herbage is brittle, but it is possible that fragmentation occurs between cells without rupturing them as has been observed in studies of forage grasses (Wright and Vincent, 1996). Microscopic evaluation is required to evaluate this.

- More detailed assessment of what fractions of N (protein-N vs. NPN) are released during comminution would benefit understanding of circadian patterns of rumen N metabolism.
- The studies conducted here examined N release during comminution, and suggested possible impacts on subsequent ruminal N metabolism and excretion. *In vitro* or *in vivo* studies to explore the effects of feeding diets grown under varying N fertiliser regimes would provide credibility to outcomes.
- The negative implications for applying fertiliser to lucerne swards warrants investigation concerning the N fractions in herbage, and effects of N fertilisation on N release from lucerne during maceration when it is grown in a multi-species, rather than a monoculture sward.

Appendix A

Detailed methodologies

A.1 Method for measuring the toughness of herbage

1. Connect the mincer, logger and laptop.

   Connect the power source to the logger at point **A** (see photo below) & turn power on at wall.

   Connect the mincer power cord at point **B**.

   Connect the logger to the laptop with a USB cord at point **C** & turn power on at wall.

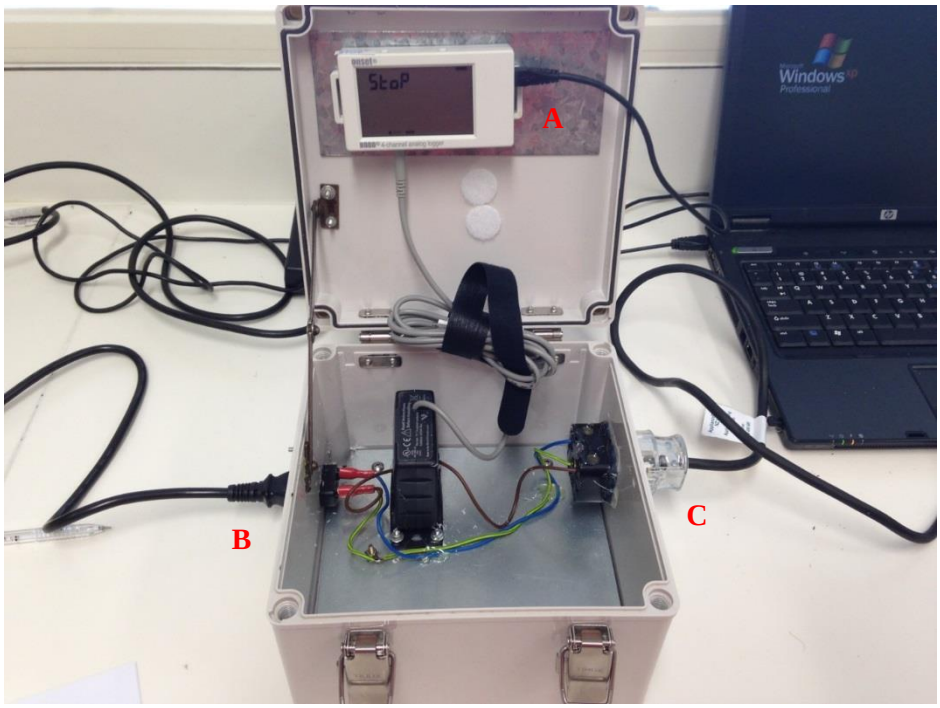


Plate A.1.1 Configuration of connected logger.

2. Launch the HOBO® analogue logger (Onset Computer Corporation, MA, USA) software installed on the laptop, by opening the program. A window will appear, select 'Device' then 'Launch' from the toolbar.
3. A second window will appear allowing the sensor to be configured and settings defined. Set the logging mode to be 'Fixed interval' at '1 second' intervals, with the start and stopping of logging to occur when the start button is pressed.
4. The logger is ready to record when the screen looks like this:

Plate A.1.2 Logger screen

5. Set up the mincer. Insert the mincer head into the body of the machine and lock it into place.
6. Insert the mincer attachments into the head in the sequence as follows:

Plate A.1.3 Logger screen configuration of mincer attachments.

7. The head of the mincer must stay cold so that the sample does not thaw out. Thawed samples will not mince. An ice-box attachment was developed to do this and is inserted over the mincer head and filled with dry and crushed ice. Place enough dry ice in the bottom of the dry ice to cover the bottom with about 20 mm of dry ice, fill the rest of the ice box with crushed ice leaving a further 20 mm gap at the top so a final layer of dry ice can be added. The ice box cannot be filled with dry ice entirely, this will cause the sample and mincer to freeze solid, and malfunction.
8. Place the lid on the icebox and the sample tray on the mincer.

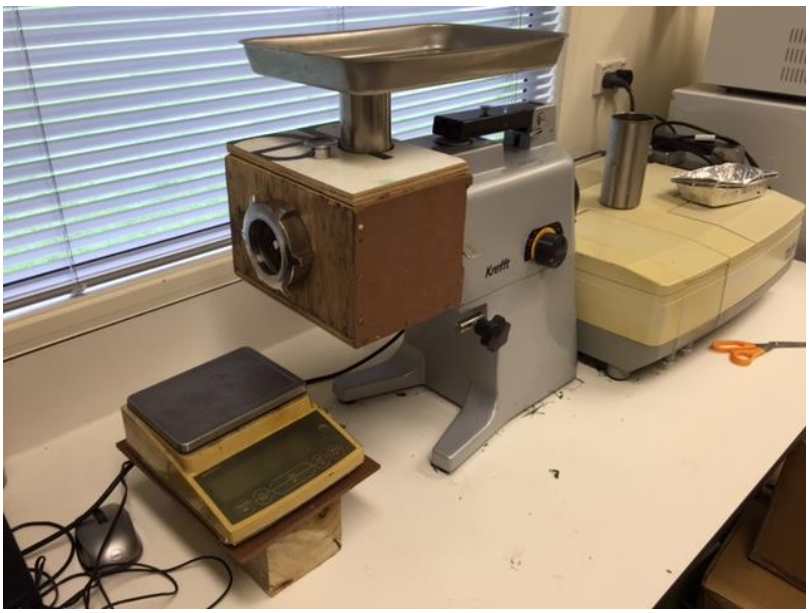


Plate A.1.4 Mincer with ice box and sample tray attached.

9. To start logging, press the start/stop button.
10. When the mincer is first turned on it will initially draw a peak of energy and then settle after about two minutes of running. After this it can be turned off and then on again and it will settle to an energy consumption of around 0.014 amps. Therefore, make sure the mincer has been run for several minutes before samples are put through the mincer.
11. To measure the energy required to macerate forage samples, the samples must be prepared for mincing by first freezing a large (~ 2 kg) subsample of fresh herbage at -20°C, then chopping the material with a guillotine to 20 mm long fragments. Care must be taken during chopping so that the material does not thaw.

Plate A.1.5 Chopping herbage sample using a guillotine.

12. Development of the methodology suggested that different forages had different base loads. That is, when a sample of the forage is fed into the mincer, the baseload is the energy the mincer is consuming when the mincer head is full of sample and the mincer is turned on. Therefore, it was determined that a baseload measurement should be taken at the start of every batch of samples measured, and each batch would contain like samples.
13. To calculate the amount of energy consumed during maceration. The sample was fed into the mincer at a constant rate until sample was flowing out of the mincer. The machine is turned off, then turned on for at least 120 s to obtain a baseload measurement.
14. The machine is restarted, and the remainder of the sample is fed into the mincer (again, at a constant rate). This is your measurement of energy consumption for that sample. Once all sample has gone into the mincer, the machine is turned off and wet weight of the sample that has passed out of the mincer is recorded.
15. Feed the next sample into the mincer until approximately 50 g of material has come out of the mincer. This is to "purge" the mincer of the previous sample.
16. Feed the remainder of the sample into the mincer, record the wet weight as above.
17. Repeat for a third sample, then take another baseload reading.
18. After six samples have been processed (minced), download the data from the logger by selecting 'Readout' from the 'Device' tab in the toolbar.
19. Export the data into an excel spreadsheet.

20. For each block of three samples, calculate the average baseload energy requirement from the baseload readings at the start and end of the three samples.
21. Subtract this average baseload reading from each data point (1 s interval) during the measurement time, then the sum of the remaining energy consumed (total energy – baseload energy) is the amount of energy consumed to mince the wet weight of forage material fed into the mincer.
22. Calculate the amps required to mince 1 g of wet material:

$$Amp/g\ WW = WW / SA$$

23. Calculate the amps required to mince 1 g of dry material using the DM% determined previously:

$$Amp/\ g\ DW = SA /((WW / 100) \times DM)$$

Where:

SA = Sum of amps used to mince
WW = Wet weight of sample minced (g)
DM = DM % of the sample

Appendix B

Supplementary data tables

B.1 **Table B.1** Seasonal physical and chemical data of herbage from five species.

Variable	DM	Leaf	Lamina strength	Maceration energy	CP[1]	NDIN[2]	WSC[3]	NDF[4]	ADF[5]	Lignin	Ash
Units	%	%	Newtons/mm^2	J g/FW	----------g/ 100g ----------						
Chicory											
Summer	10.5	100.0	0.63	17.6	12.9	0.9	12.9	18.2	13.5	2.8	16.2
Autumn	8.0	100.0	0.35	26.6	17.6	2.9	13.8	18.6	14.2	3.1	15.9
Winter	12.4	98.3	0.80	47.1	12.1	0.9	16.5	17.6	12.6	2.6	14.0
Spring	9.8	88.5	0.42	30.4	14.4	2.5	14.1	19.5	14.5	2.6	16.6
Lucerne											
Summer	26.6	55.1	0.47	113.1	21.4	1.1	9.8	30.5	26.4	5.6	9.7
Autumn	14.6	45.5	0.33	70.1	28.7	2.0	8.2	25.2	20.6	4.3	9.9
Winter	16.1	47.7	0.51	57.6	30.4	1.6	10.0	23.0	18.7	3.6	9.9
Spring	15.4	43.2	0.35	76.5	26.0	1.3	9.0	28.2	23.3	4.5	10.0
Plantain											
Summer	17.3	75.5	0.65	67.6	8.9	2.8	14.1	33.5	30.0	7.9	12.8
Autumn	12.4	99.6	0.76	46.9	13.7	5.7	16.4	22.6	18.5	4.3	15.3
Winter	13.3	98.4	0.66	53.5	11.7	1.4	18.2	18.2	15.3	3.5	13.6
Spring	10.7	68.9	0.56	48.9	15.9	2.2	14.2	24.4	19.1	3.9	14.3
Perennial ryegrass											
Summer	24.7	79.8	1.06	191.6	20.2	4.8	11.3	47.2	26.6	3.9	10.8
Autumn	19.3	82.6	0.99	150.5	17.6	4.1	14.6	44.8	25.9	2.4	11.3
Winter	20.5	93.4	1.32	137.0	15.7	3.7	23.1	39.1	22.4	1.5	9.3
Spring	22.2	78.9	1.11	170.4	13.6	3.3	23.0	40.3	23.1	1.9	9.3
White clover											
Summer	21.6	54.3	0.72	49.4	24.6	1.2	13.4	21.7	15.1	3.3	9.6
Autumn	14.5	67.4	0.48	36.1	28.6	1.7	10.9	20.1	13.3	2.8	8.7
Winter	14.0	67.5	0.51	42.7	28.5	1.8	13.0	19.0	12.8	2.8	8.1
Spring	12.9	50.4	0.48	42.4	25.1	1.3	10.8	22.1	15.9	3.4	10.3

[1]CP, Crude protein; [2]NDIN, Neutral detergent insoluble nitrogen; [3]WSC, Water-soluble carbohydrate; [4]NDF, Neutral detergent fibre; [5]ADF, Acid detergent fibre.

B.2 **Table B.2**. Absolute release (g/100 g DM) of crude protein (CP) and water soluble carbohydrates (WSC) following maceration of herbage from five species during four seasons, and the average of all seasons for each species.

	Combined		Summer		Autumn		Winter		Spring	
Species	WSC	CP	WSC	CP	WSC	CP	WSC	CP	WSC	CP
Chicory	13.1^{b}	2.9^{c}	11.8^{a}	1.0^{b}	12.7^{b}	6.2^{ab}	15.7^{b}	1.0^{c}	12.6^{b}	3.1^{bc}
Lucerne	8.0^{d}	11.5^{a}	8.4^{b}	9.5^{a}	6.2^{d}	10.4^{a}	9.1^{c}	13.7^{a}	8.1^{d}	11.6^{a}
Plantain	14.3^{ab}	0.8^{d}	12.3^{a}	0.2^{b}	15.3^{a}	0.9^{c}	17.0^{ab}	0.3^{c}	12.4^{b}	1.3^{c}
Perennial ryegrass	15.7^{a}	6.2^{b}	8.8^{b}	7.7^{a}	12.8^{b}	5.4^{b}	20.8^{a}	5.6^{b}	20.3^{a}	4.9^{b}
White clover	10.5^{c}	6.3^{b}	12.3^{a}	8.7^{a}	9.3^{c}	4.9^{bc}	11.1^{c}	6.1^{b}	9.4^{c}	5.7^{b}
SED	0.7	0.6	1.1	1.8	0.9	1.8	1.7	0.5	0.5	1.1
p-Value	< 0.001	< 0.001	0.037	0.007	< 0.001	0.023	0.001	< 0.001	< 0.001	< 0.001

Means in columns sharing the same lower case superscript are not significantly different.

B.3 **Table B.3** Morphological composition of herbage from four forages fertilised at four rates of nitrogen in autumn and spring.

			N Fertiliser application rate (kg N/ha/y)						*p*-Value		
			0	100	200	350	Mean	SED	N rate	Forage	N × Forage
Leaf content (%)											
	Autumn										
		All	78	84	81	81	81	5.9	0.808	< 0.0001	0.9996
		Chicory	99	102	100	94	99^{AB}	15.4	0.9677		
		Lucerne	50	55	48	56	52^{D}	13.9	0.9166		
		Plantain	94	100	100	100	100^{A}	12.1	0.9198		
		P. ryegrass	75	87	83	86	83^{BC}	10.7	0.6499		
		White clover	72	73	71	67	71^{CD}	14.0	0.9757		
	Spring										
		All	65	62	65	73	66	2.2	0.0002	< 0.0001	0.1136
		Chicory	85	90	88	98	90^{A}	5.4	0.2008		
		Lucerne	45	40	39	46	43^{D}	5.0	0.3505		
		Plantain	65	65	71	77	69^{B}	4.9	0.0499		
		P. ryegrass	71^{bc}	64^{c}	79^{ab}	85^{a}	75^{B}	4.5	0.0004		
		White clover	60	51	51	57	55^{C}	4.6	0.1803		
Stem (%)											
	Autumn										
		Lucerne	39	36	40	36	38	2.3	0.2012		
	Spring										
		All	20	27	24	19	23	3.7	0.2088	< 0.0001	0.8850
		Chicory	5	6	8	5	6^{C}	9.0	0.9918		
		Lucerne	40	46	48	43	45^{A}	8.3	0.7967		
		Plantain	23	31	28	21	26^{B}	8.1	0.5658		
		P. ryegrass	22	31	17	11	20^{B}	7.5	0.0820		
		White clover	11	21	22	18	18^{B}	7.7	0.5629		

Means in rows sharing the same lower case superscript are not significantly different; Means in columns sharing the same upper case superscript are not significantly different.

B.4 **Table B.4** Correlation (R^2) between force to punch, maceration energy, water soluble carbohydrate (WSC) and crude protein (CP) release and physcical and chemical characteristics of herbage.

	Autumn				Spring			
	Chicory	Lucerne	Plantain	Perennial ryegrass	Chicory	Lucerne	Plantain	Perennial ryegrass
Herbage strength								
DM%	**0.75**	0.07	0.10	**0.71**	**0.74**	0.08	0.02	**0.83**
NDF	0.10	**0.47**	0.16	**0.63**	0.00	0.08	**0.35**	**0.62**
Herbage toughness								
DM%	**0.83**	0.12	**0.56**	0.10	**0.79**	0.26	**0.65**	**0.40**
NDF	0.03	0.06	0.04	0.02	0.04	0.10	0.01	**0.88**
CP release								
DM%	0.27	***0.65***	***0.64***	0.26	0.08	0.22	0.10	***0.85***
NDF	0.05	0.05	0.06	0.10	0.02	0.00	0.15	***0.59***
CP	**0.52**	0.30	**0.34**	**0.61**	0.00	0.03	0.04	**0.76**
Nitrate	0.28	0.00	**0.51**	0.31	0.09	0.05	0.08	**0.67**
Herbage strength	***0.72***	0.02	***0.58***	**0.53**	0.29	0.01	0.09	***0.89***
Herbage toughness	***0.43***	0.08	***0.44***	0.24	0.18	0.14	0.10	***0.46***

Values in bold are significant at the 5% level, bold values denote a positive relationship, bold italicised values denote a negative relationship.

B.5 **Table B.5** Distribution of particle fractions (on a DM basis) of macerated material by sieve size from five species.

		N Fertiliser application rate (kg N/ha/y)						*p*-Value		
		0	100	200	350	Mean	SED	N rate	Forage	N × Forage
Autumn	4 + 2 mm								< 0.001	0.156
	Chicory	19.4	25.5	21.7	21.5	22.0^{A}	4.47	0.568		
	Lucerne	22.8	22.7	21.0	24.7	22.8^{A}	3.78	0.803		
	Plantain	11.0	14.8	12.6	12.4	12.7^{B}	3.24	0.686		
	Perennial ryegrass	22.5	22.1	26.7	25.3	24.1^{A}	4.34	0.545		
	1 + 0.5 + 0.075 mm								< 0.001	0.096
	Chicory	40.8	38.3	42.8	42.4	41.1^{B}	3.67	0.464		
	Lucerne	45.6	42.2	47.0	47.1	45.5^{AB}	3.11	0.382		
	Plantain	48.9	48.7	49.5	49.7	49.2^{A}	2.66	0.968		
	Perennial ryegrass	43.8	43.3	39.3	41.8	43.3^{AB}	3.57	0.036		
	Soluble								0.477	0.682
	Chicory	39.9	36.1	35.5	36.1	36.9	3.93	0.768		
	Lucerne	31.6	35.1	32.1	28.3	31.8	3.33	0.246		
	Plantain	40.1	36.4	37.9	37.8	38.1	2.85	0.681		
	Perennial ryegrass	33.7	34.6	34.0	33.0	35.1	3.82	0.298		
Spring	4 + 2 mm								< 0.001	0.343
	Chicory	21.5	23.6	21.7	24.9	22.9^{A}	4.64	0.882		
	Lucerne	28.5	23.6	23.3	21.7	24.3^{A}	4.23	0.460		
	Plantain	15.6	14.3	16.7	14.9	15.4^{B}	4.12	0.943		
	Perennial ryegrass	27.1^{a}	17.2^{b}	16.5^{b}	20.2^{b}	20.3^{AB}	3.80	0.034		
	1 + 0.5 + 0.075 mm								< 0.001	0.144
	Chicory	43.9	38.3	36.2	41.7	40.0^{B}	4.89	0.330		
	Lucerne	42.6	40.5	40.4	47.1	42.7^{B}	4.46	0.344		
	Plantain	50.5	48.8	57.6	51.5	52.1^{A}	4.34	0.225		
	Perennial ryegrass	35.1	39.8	42.1	36.3	38.3^{B}	4.01	0.294		
	Soluble								0.001	0.104
	Chicory	34.6	38.1	42.0	33.4	37.0^{AB}	4.58	0.226		
	Lucerne	29.0	35.9	36.4	31.2	33.1^{B}	4.17	0.255		
	Plantain	34.0^{ab}	36.8^{a}	25.7^{b}	33.5^{ab}	32.5^{B}	4.07	0.058		
	Perennial ryegrass	37.8	43.0	41.4	43.6	41.5^{A}	3.75	0.410		

Means in rows sharing the same lower case superscript are not significantly different; Means in columns sharing the same upper case superscript are not significantly different.

B.6 **Table B.6** Variation in boli characteristics between cows, and effect of age on boli characteristics.

	Cow								Maturity			
	1	2	3	4	5	6	SED	*p*-Value	Young	Mature	SED	*p*-Value
Age of cow (y)	4	4	4	10	10	10						
Fresh weight of boli (g)	182.6[a]	147.4[b]	168.9[ab]	171.2[ab]	157.5[ab]	172.2[ab]	8.2	0.020	166.8	164.9	6.3	0.794
Dry weight of boli (g)	11.7	10.5	12.7	13.1	12.2	12.4	1.1	0.317	11.6	12.6	0.7	0.293
Feed content of boli (g)	104.1	97.7	118.1	113.0	114.0	116.0	9.5	0.273	106.5	114.8	5.5	0.271
Saliva content of boli (g)	78.2[a]	50.0[b]	51.1[b]	57.9[b]	43.2[b]	55.9[b]	5.4	0.001	60.4	49.7	4.6	0.145
Saliva added per 100 g feed (g)	75.9[a]	51.4[ab]	44.5[b]	53.5[ab]	41.4[b]	55.1[ab]	8.5	0.027	58.1	47.2	5.6	0.191

Means in rows sharing the same lower case superscript are not significantly different.

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