In sacco digestion kinetics of plantain and ryegrass-white clover harvested in the morning and afternoon

LA Box*, GR Edwards and RH Bryant

Faculty of Agriculture and Life Sciences, PO Box 85084, Lincoln University, Lincoln 7647, Christchurch

**Corresponding author.* Email: lisa.box@lincolnuni.ac.nz

Abstract

The objective of this experiment was to compare rumen degradation characteristics of ryegrass (*Lolium perenne*), white clover (*Trifolium repens*) or plantain (*Plantago lanceolata*) harvested either in the morning (0700) or afternoon (1600). Fresh herbage material from each treatment was weighed into duplicate nylon bags and incubated in four lactating Jersey × Friesian dairy cows with rumen fistula. Differences in nutritive value between forage types was greater than effect of harvest time as was the effect on rumen degradation characteristics. Plantain had faster (P<0.001) DM disappearance rates than perennial ryegrass-white clover (12.2 vs 8.80 %/hr). The soluble fraction was greater (P=0.001) for forages harvested in the afternoon (25.2% of DM) than those in the morning (15.4% of DM) regardless of forage type. Nitrogen degradation was almost complete (>95% of N) and similar for all treatments (P>0.05). An interaction for effective degradability revealed there was no effect of harvest time on DM and OM for plantain. However, perennial ryegrass-white clover harvested in the afternoon has a greater effective degradability of DM and OM than that harvested in the morning. This suggested there is a larger influence of harvest time on some degradation characteristics of perennial ryegrass-white clover compared with plantain.

Keywords: diurnal; herb; *Lolium perenne*; *Plantago lanceolata*; rumen degradation; *Trifolium repens*

Introduction

In New Zealand dairy systems alternative forage types to perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) (RWC) pastures such as plantain (*Plantago lanceolate*) are increasing in popularity. Plantain is a herb species which can produce at least as much dry matter (DM) as traditional RWC pastures (Moorhead et al. 2009; Martin et al. 2017). There is also evidence of greater milk production by cows grazing plantain (Box et al. 2016; Pembleton et al. 2016). Pembleton et al. (2016) reported improved milk production by cattle grazing a mixed sward of RWC and plantain (at 50% herbage dry matter intake (DMI)) compared with those grazing RWC alone in early lactation. Box et al. (2016) found cows grazing pure plantain swards produced 11% more milk than those grazing RWC in late lactation. The increase in milk production was attributed to an increase in DMI despite similar allocations.

One of the mechanisms that controls herbage DM intake is rumen fill (Allen 2014). Forages such as RWC, with a relatively large high content, are more slowly digested compared with forages, such as plantain, which has a low fibre content (John et al. 1987; Burke et al. 2002; Chaves et al. 2006). Fibre concentration, DM content and digestibility of forages show diurnal fluctuations. For example, RWC and plantain harvested in the afternoon had a lower fibre content than did herbage harvested in the morning (Cosgrove et al. 2009; Box et al. 2017). Changes in fibre content are offset by changes in other variables such as sugars and/or crude protein (CP) which can also influence degradation rate via availability of substrates for microbes. Mechanisms which lower the fibre content of forages may be useful to increase the rate of degradation and in turn improve herbage DM intake.

The rate and extent of degradation of DM, organic matter (OM) and nitrogen (N) in the rumen has implications on animal production and nitrogen use efficiency. Adequate balance of OM relative to N available in the rumen has the potential to improve microbial protein synthesis and increase metabolisable protein supply to the ruminant (Tas et al. 2006). Therefore, alternative forages, such as plantain, or diurnal allocation strategies which improve the proportion of OM degraded relative to N, may be beneficial in improving nitrogen use efficiency in dairy cows. The objective of this research was to determine the DM, OM, and N digestion kinetics of plantain and RWC herbage harvested in the morning and afternoon.

Materials and methods

Experimental design

Rumen degradation characteristics were determined by nylon bag incubations of plantain and RWC herbage. The experiment was a 2 × 2 factorial design. There were two pasture treatments of either plantain or RWC which were harvested at two times of the day; 0700 or 1600 h. Fresh herbage material of each treatment was weighed into duplicate nylon bags and incubated in four rumen-cannulated multiparous Jersey × Friesian lactating dairy cows on a pasture diet of spatially separate RWC (50%) and plantain (50%). Each cow received one treatment per run, there were four runs, so all cows received each treatment. The experiment was carried out at the Lincoln University Research Dairy Farm with the approval of the Lincoln University Animal Ethics Committee (AEC 398) in April 2016.

Forage preparation

Herbage for rumen incubations was collected from

existing paddocks sown with either plantain (cv. Tonic) or perennial ryegrass (cv. Arrow) and white clover (cv. Kopu II). Perennial ryegrass-white clover pastures were established in March 2014 and the plantain pastures in December 2014. Perennial ryegrass-white clover for incubation consisted of 89% perennial ryegrass and 11% white clover and plantain samples consisted of 100% plantain leaf. For each treatment, four bags of herbage of approximately 1 kg each were harvested to grazing height using hand shears. Harvested RWC herbage was cut to ~2 cm lengths and plantain to approximately 2 cm^2 to replicate mastication. Herbage of approximately 40 g dry weight was weighed into individual dacron bags (10 x 15 cm with 50µ pore size). Bags of each treatment were attached to a 1 m galvanised chain and frozen at -16°C until required. A total of 16 chains were prepared, to represent each treatment for four runs using the four cows. Duplicate and triplicate for the 48 h interval - bags were attached to the chain using plastic cable ties (total 13 bags per chain). Two subsamples of herbage for each treatment were taken. The first was oven dried at 65°C for 48 h to determine DM % and the second subsample was freeze dried and ground through a 1 mm sieve. Ground material was NIRS scanned to determine chemical composition calculated from forage samples in Martin et al. (2017).

Rumen incubations

Removal of *in sacco* bags commenced at 0630 h between 11 and 23 April 2016 using incubation intervals of 3, 6, 9, 12, 24, and 48 h, including wash samples at 0 h. Following each incubation, duplicate bags were plunged into ice water and rumen debris removed, then washedby hand until water ran clear, then dried at 65°C for 48 hours. Total DM was recorded for each bag and subsamples were analysed for OM (ashed at 500 °C) and N (Elementar Vario MAX) concentration.

Digestion kinetics

Rumen factorial disappearance rate of DM, N, and OM was calculated using a nonlinear model based on equation 1. Effective degradability of DM, N, and OM was calculated using equation 2 fitted to constant rumen passage rate of 0.06 %/h.

Equation 1. Factorial disappearance, where P= disappearance, a= soluble fraction, b= potentially degradable fraction, e= exponential, c= fractional degradation rate (%/ hour), and t= time (hour) (Ørskov et al. 2009)

$$P = a \times b \times (1 - e^{-ct})$$

Equation 2. Effective degradation (ED), where P= disappearance, a= soluble fraction, b= potentially degradable fraction, c= fractional degradation rate (%/ hour), and k= rumen passage rate (%/hour) (Valderrama et al. 2011)

$$ED = a + \frac{b \times c}{c + k}$$

Equation 3. Effective rumen degradable protein (ERDP), where P= disappearance, a= soluble fraction, b= potentially degradable fraction, c= fractional degradation rate (%/hour), k= rumen passage rate (%/hour),CP=crude protein (%) and =efficiency of degradable protein (0.8) (Valderrama et al. 2011)

$$ERDP = a + \alpha \frac{b \times c}{c + k} \times CP$$

Statistical analysis

A two-way ANOVA (GenStat 15.1) was used to analyse the chemical composition of forages and parameter estimates to determine effects of forage type, harvest time and there interaction. Forage type and harvest time were fixed terms and cows were replicates for the degradation parameters estimated from the non-linear fits.

Results

The chemical composition of plantain and RWC harvested in the morning and afternoon is shown in Table 1. The proportion of fibre (NDF) was 36% lower (P<0.001) in plantain than RWC. The effect of time of day on the fibre content of herbage was greater for RWC than plantain. The NDF concentration of pasture was reduced by 8% by harvesting in the afternoon. The diurnal increase in DOMD was twice as large (P=0.047) for RWC than for plantain (+5.7 g/kg DM vs. +3.0 g/kg DM). While the difference in DOMD between plantain and RWC harvested in the morning was significant (P=0.013), the difference was small (0.5%). The DM% of plantain was two thirds that of RWC and had a smaller diurnal increase.

DM digestion kinetics

The *in sacco* DM disappearance data are illustrated in Figure 1a. From hours 6 to 24 after incubation, DM disappearance was more rapid (P<0.001) for plantain than for RWC. DM disappearance tended to be greater for pasture harvested in the afternoon than morning until 24 hours after incubation. Estimated DM fractions and degradation parameters are summarised in Table 2. The percentage of DM released during hand washing of un-incubated samples (soluble DM; fraction A) was greater for samples cut in the afternoon regardless of forage type. The fractional DM degradation rate per hour was greater for plantain compared with RWC. There was no effect of harvest time on the fractional degradation rate of DM per hour.

OM digestion kinetics

There was little effect of pasture type or time of day on the *in sacco* disappearance of OM (Figure 1b). The soluble fraction of OM was greater for samples harvested in the afternoon and was not different between forage types (Table 2). There was no effect of forage type or time of harvest on fractions a, b or c or the potential degradability of OM. The effective degradability was greater for plantain (average 67.3%) than for RWC (61.2%). Ryegrass-white clover harvested in the afternoon had a greater effective degradability of 64.2% than did RWC harvested in the

Table 1 Forage dry matter (DM) content (%), chemical composition (% of DM) and predicted metabolisable enery (ME) (MJ ME/kg DM) determine by near infrared reflectance spectroscopy of plantain and perennial ryegrass-white clover harveted at 0700 (AM) or 1600 h (PM).

	Plantain		Ryegrass-white clover			Significance		
	AM	PM	AM	PM	SEM	Forage	Time	F*T
DM	8.5	11.6	12.7	19.1	1.04	<0.001	<0.001	<0.001
ADF	21.3	21.3	24.2	21.2	0.648	<0.001	<0.001	<0.001
WSC	14.9	20.2	8.4	13.2	2.11	<0.001	<0.001	0.623
DOMD	73.4	76.4	73.9	79.6	1.22	0.013	<0.001	0.047
ME	11.7	12.2	11.8	12.7	0.195	0.013	<0.001	0.047
NDF	26.5	25.7	42.6	39.2	3.74	<0.001	<0.001	0.012
OM	86.1	87.2	91.2	91.0	1.13	<0.0013	0.032	0.003
CP	27.5	24.6	25.3	27.1	0.587	0.596	0.058	<0.001

Table 2 Mean dry matter (DM), organic matter (OM) and nitrogen (N) degrdation characteristics of ryegrass-white clover and plantain harvested at 0700 (AM) or 1600 (PM) defined as solube fraction (a), potentially soluble fraction (b), fractional dissapearnce rate (c), potentially degradable (PD), effective degradability at 0.06 h (ED) and effective rumen degradable protein (ERDP).

	Plantain		Ryegrass-white clover			Significance		
%	AM	PM	AM	PM	SEM	Forage	Time	F*T
Dry matter								
a	16.4	23.7	14.4	26.6	0.019	0.886	0.010	0.425
b	74.8	68.7	77.0	64.5	0.022	0.795	0.032	0.409
c (/hour)	12.5	11.9	8.01	9.58	0.008	0.050	0.761	0.487
PD	91.3	92.4	91.4	91.2	0.995	0.827	0.859	0.785
ED	66.7	68.7	57.6	65.1	1.32	<0.001	0.003	0.047
Organic matter								
a	20.5	24.5	17.4	26.8	0.018	0.891	0.090	0.452
b	68.5	65.8	74.0	63.0	0.076	0.664	0.121	0.318
c (/hour)	10.3	10.1	7.66	9.36	0.009	0.439	0.726	0.662
PD	88.9	90.3	91.4	89.9	0.954	0.576	0.968	0.529
ED	66.5	68.0	58.2	64.2	1.35	<0.001	0.005	0.043
Nitrogen								
a	2.4	10.3	14.6	20.2	0.035	0.005	0.049	0.704
b	93.9	85.5	80.9	75.3	0.040	0.042	0.188	0.784
c (/hour)	8.84	8.56	7.09	8.27	0.006	0.324	0.656	0.470
PD	96.3	95.8	95.5	95.5	1.14	0.846	0.931	0.938
ED	58.0	60.0	57.8	61.8	1.23	0.608	0.086	0.530
ERDP	151	136	151	162	3.46	0.004	0.575	0.006

morning (58.2%). There was no time-of-harvest effect on plantain samples.

N digestion kinetics

From hours 6 to 24 after incubation, N loss was greater (P<0.001) for plantain than for RWC, and greater for pastures harvested in the afternoon than those harvested in the morning (Figure 1c). The soluble and potentially soluble fraction of N was greater (P<0.05) for plantain than RWC (Table 2). Forages harvested in the afternoon had a greater soluble fraction of N than those harvested in the morning. There was no effect of forage type or time of day on the fractional degradation rate, potential degradability or effective degradability of N.

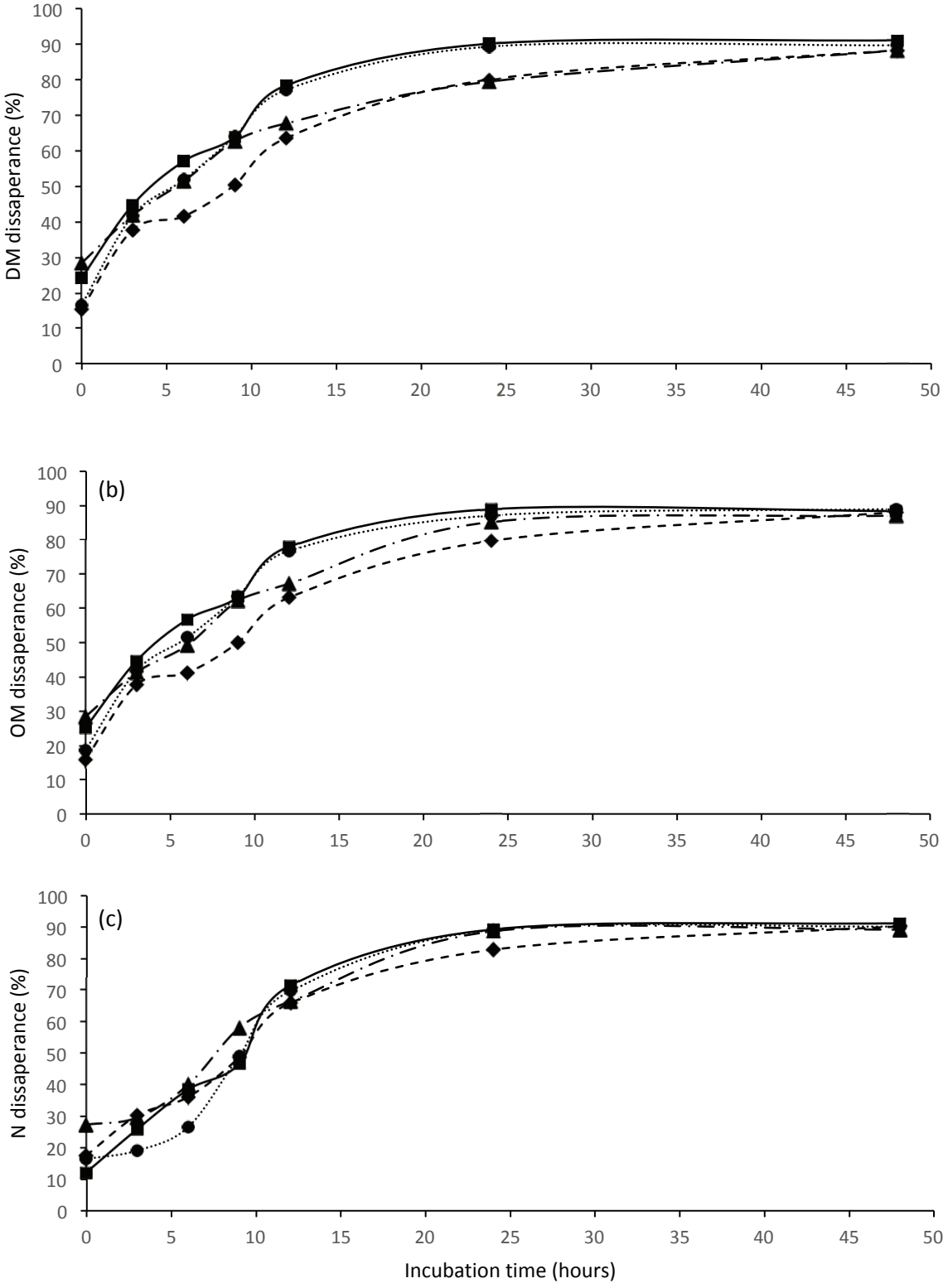
Discussion

This experiment compared the *in sacco* digestion kinetics of plantain and RWC harvested in the morning or afternoon to ascertain if digestion of forages was altered by management practises such as time of forage allocation. An interaction between forage and time revealed the effect of time of day on chemical composition and effective degradation of OM and DM was greater for RWC than for plantain. However, the effect of time of day on other degradation parameters was similar between forages.

Forage effect

In the current experiment, DM disappearance between 6 and 24 h, was more rapid for plantain than for RWC resulting in overall greater DM degradation rate. Although degradation rates in this experiment were much slower than those reported by Burke et al. (2000), the trend for plantain to have a faster rumen clearance time (25%/hr) compared with perennial ryegrass (11%/hr) was similar. Burke et al.

Figure 1 *In sacco* disappearance of dry matter (DM) (a), organic matter (OM) (b) and nitrogen (N) (c) for ryegrass-white clover and plantain harvest at 0700 (AM) or 1600 (PM). Error bars are ±SEM. Plantain-AM(⋯●⋯), plantain-PM(—■—), RWC-AM (--◆--), RWC-PM (- -▲- -).



(2000) attributed the increased rumen clearance time of plantain to the structure of the plant cells and their rumen degradation characteristics. Plantain is known to contain significant concentrations of pectin, a structural but readily fermentable carbohydrate (Judson et al. 2009). These results support plantain as a mechanism to improve rumen clearance times via increased DM disappearance rates, which may be useful to increase herbage DM intake.

Cows grazing pastures which include plantain have consistently shown lower urinary N concentrations compared with those grazing RWC pastures (Box et al. 2016; Bryant et al. 2017; Minneé et al. 2017). Minneé et al. (2017) suggested the lower CP released from plantain in the early stages of *in sacco* degradation compared with perennial ryegrass may have contributed to lower urinary N concentration. In the current experiment the soluble fraction (A) of N was greater in RWC compared with plantain, which was similar to the results found by Minneé et al. (2017). However, there was no difference in the rate of degradation of N between RWC and plantain. Further, from 6 to 24 hours after incubation, the proportion of N which had disappeared from plantain was greater than that from RWC which was unexpected. Unlike Minneé et al. (2017) the N content of plantain and RWC was similar. Therefore, the greater N disappearance of plantain compared to RWC in this study was due to the increased rate of DM disappearance. For example, from hours 6, 9, 12 and 24, more DM had disappeared from plantain than from RWC, which coincided with the greater N loss.

Time-of-day effects

Forage type had a greater effect on the effective degradability and rate of degradation of DM and OM. However, the time of day that forages were harvested appeared to have a greater effect on the A and B digestion fractions of DM. Diurnally, there was an increase in the A fraction of DM for RWC and plantain, and a reduction in the B fraction. This meant that overall there was no time-of-day effect on the potentially degradable DM of RWC or plantain. This result was not surprising given there were only small increases in WSC of herbage and, therefore, small reductions in NDF. In this experiment the concentration of WSC was 5.3% DM higher for RWC and 4.9% DM higher for plantain harvested in the afternoon than for forages harvested in the morning. The magnitude of the difference was smaller than that reported by Cosgrove et al. (2007) of 6.1% DM for autumn harvested perennial ryegrass but similar to results found in a previous experiment at the same site. Box *et al*. (2017) found the diurnal increase in WSC for ryegrass was on average 5.8% DM greater for afternoon harvested perennial ryegrass and 2.9% DM greater for afternoon harvested plantain than forages harvested in the morning.

Despite no difference in the potential degradability of OM or DM between forage cut in the morning and afternoon, the effective degradability was improved when RWC and plantain were harvested in the afternoon. This diurnal effect was greater for RWC than plantain. This was likely due to RWC having larger changes in chemical composition diurnally. Notably the diurnal reduction in fibre was greater for RWC than that for plantain. This is similar to results found by Box et al. (2017), where there was a greater diurnal change in the nutritive value of perennial ryegrass, than plantain. Management strategies which allow allocation of RWC in the afternoon and plantain in the morning may provide benefits in milk production, through increased DM intake.

Conclusions

The results showed that different forages have different degradation parameters and these are influenced by diurnal effects on the relative chemical composition. Plantain degrades faster than RWC, which resulted in faster N degradation rates. As a result the use of plantain as an alternative forage for dairy systems may be useful to increase voluntary herbage DM intake. Effective degradability of DM and OM was improved when RWC and plantain were harvested in the afternoon. This diurnal effect was greater for RWC than for plantain.

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