Apparent digestibility and rumen fermentation of fresh plantain (*Plantago lanceolata* cv Ceres Tonic) and perennial ryegrass (*Lolium perenne* cv Nui) -based pasture fed to red deer (*Cervus elaphus*)

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ABSTRACT

The aim of this study was to compare the apparent digestibility and rumen fermentation products in deer fed plantain (*Plantago lanceolata*) and perennial ryegrass-based pasture (*Lolium perenne*) during spring. Forages were cut and fed fresh in a cross-over design to seven castrated, rumen fistulated red deer (*Cervus elaphus*) housed individually indoors in metabolism crates. Plantain contained greater levels of organic matter and lignin and had a greater ratio of readily fermentable to structural carbohydrates and lower levels of crude protein ($P < 0.01$), compared with ryegrass-based pasture. The apparent digestibility of organic matter for both forages was high, but less for plantain (74.9%) compared with ryegrass-based pasture (81.5%) ($P < 0.01$). The ammonia concentration of rumen fluid from deer fed plantain (4.6 mmol/L) was less compared with fluid from deer fed ryegrass-based pasture (20.9 mmol/L) ($P < 0.01$). The apparent reduction in degradation of protein to ammonia in the rumen of deer fed plantain may have implications for increasing the efficiency of use of dietary protein in grazing ruminants. Further research is required to investigate the effect of feeding plantain, and its secondary compounds, on the rumen microbial ecosystem and rumen fermentation.

Keywords: Apparent digestibility; plantain; pasture; red deer; rumen fermentation.

INTRODUCTION

Deer, similar to sheep and cattle in New Zealand (NZ), are normally grazed year-round on perennial ryegrass (*Lolium penne*) dominant swards containing less than 20% white clover (*Trifolium repens*) and other pasture species (Ramírez-Restrepo & Barry, 2005). Pastoral based livestock production is low cost, however, the nutritive value of pastures is highly variable (Woodfield & Easton, 2004). To improve the consistency and overall nutritive value of pasture and the sustainable productivity of grazing ruminants, novel forages, i.e. herbs and legumes, have been introduced either as a component of ryegrass-based pasture mixes or as specialist forage crops.

When young deer were grazed on red clover, lotus and chicory, respectively, growth rates were up to 27%, 22% and 47% greater than those of corresponding deer grazing ryegrass-based pasture (Barry *et al.,* 2002). Superior feeding value of these forages has been attributed to greater digestibility, voluntary feed intake (VFI), rapid rumen particle breakdown, lower rumen mean retention time, and secondary plant compounds altering rumen fermentation (Hoskin *et al.,* 1995; Kusmartono, *et al.,* 1997).

Narrow-leaved plantain (*Plantago lanceolata*) is a new forage herb suitable for deer production due to a seasonal growth pattern, like chicory, aligned with deer feed requirements (Stewart, 1996; Barry, 1998). However, anecdotal evidence suggests that plantain exhibits greater persistence under grazing than chicory. Plantain is of interest for grazing animals due to the presence of bioactive compounds (Tamura & Nishibe, 2002), a high mineral content (Hoskin *et al.,* 2003) and possible anthelmintic properties (Gustine *et al.,* 2001). Feeding value of cv Ceres Tonic plantain compared with perennial ryegrass-based pasture has been reported to be higher for lambs in summer (Moorhead *et al.,* 2002) and higher for weaner deer in autumn, but similar in spring (Hoskin *et al.,* 2005). Physical breakdown of plantain by sheep appears easier compared with perennial ryegrass (Wilman *et al.,* 1997). However, there is little understanding of the factors influencing the feeding value of forage plantain. The aim of this study is to compare the apparent digestibility and effects on rumen fermentation of plantain (cv Ceres Tonic) and ryegrass based pasture when fed to red deer.

MATERIALS AND METHODS

Experimental design

An experiment of crossover design was conducted with red deer fed either fresh ryegrass-based pasture or plantain, at the deer metabolism facility, Massey University's Deer Research Unit, September to October 2003. Factors investigated included apparent digestibility and rumen fluid composition. The six week trial consisted of two three week periods. Each period consisted of: week one, animals grazing their assigned forages; week two, animals housed individually indoors in metabolism cages (Hoskin *et al.,* 1995) whilst adapting to housing and harvested forage; and week three, the measurement period.

Animals and diet

During period one, seven adult castrated red deer (*Cervus elaphus)*, five with rumen fistulae, (mean age ± SD = 8.7 ± 6.1 years; mean live weight ± SD = 132 ± 21.1 kg) were randomly assigned to one of two forage diets, pure narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain), sown in November 2002, or permanent perennial ryegrass (*Lolium perenne* cv Nui), white clover (*Trifolium repens* cv Huia) (pasture), sown in 1992. Forage treatments were reversed for period two. Feeding occurred twice daily at 08.00 and 16.00 hours. Dry matter allowance was determined by *ad-libitum* feeding where animals were fed 1.2x the previous days DM allowance until DM intakes stabilised during week two of period one and were set at this level per animal thereafter. The dry matter content of feed offered and feed refused were determined daily by triplicate samples of approximately 200g oven dried at 100˚C for 18 hours. Dry matter intakes were determined by the DM feed offered less the DM feed refused. Botanical composition of DM offered and refused was undertaken by taking daily 200g sub-samples during the measurement week of each period, refrigerating and pooling per animal, per period. Samples per animal were homogenised, sub-sampled (~200g), then sorted into leaf, stem, clover, weed and dead matter before oven drying (100˚C for 18 hours).

Apparent digestibility and rumen fluid sampling

Feed offered, feed refused and faeces were quantitatively collected daily for seven days in week three of each period. Samples (200g) of feed offered were taken daily and pooled per period at -20˚C. Each animal's feed refused was collected, sub sampled and frozen (-20˚C) later to be pooled per period. Faeces were homogenised, sub sampled and frozen (-20˚C). Daily sub samples were later pooled per animal and per period, homogenised and triplicate sub samples (200g) taken for DM determination (100˚C 48h) and duplicate samples taken for freeze drying, grinding and chemical analysis.

Rumen fluid samples were taken at 13.00hrs on days 1 to 5 of week three of each period from the five deer with rumen fistulae. Six ml of rumen fluid was obtained following straining of rumen contents through several layers of cheese cloth for ammonia (1ml) and VFA analysis (5ml). Hydrochloric acid was immediately added to the fluid for ammonia analysis and protein precipitant to the samples for VFA analysis, samples were then centrifuged (1600 *g*) and the supernatant fluid stored at -20˚C (Domingue *et al.,* 1991).

Forage sampling and laboratory analyses

After freeze drying, all feed, refusal and faecal samples were ground to pass a 1 mm sieve (Willey Mill, USA). Samples were analysed for gross energy (GE) organic matter (OM), crude protein (CP), hot water-soluble carbohydrates (HWSC), acid and neutral detergent fibre (ADF and NDF), and lignin, according to methods described by McWilliam *et al.* (2004). Rumen fluid was analysed according to the methods of Domingue *et al.* (1991) for VFA and ammonia.

Statistical analysis

Data were analysed by SAS (SAS, 1998) using the general linear model (PROC GLM) with simple ANOVA. Type of forage (pasture or plantain), period, and their interactions were fixed effects of the model. Significance was declared at $P \leq 0.05$, and a trend was reported if $0.05 < P \leq 0.10$. All mean comparisons were by Fisher's least significant difference method after a significant main effect was detected. Standard error of the means is reported.

RESULTS

As shown in Table 1, there was found to be significant effect of forage, period and interaction of forage by period ($P < 0.05$) on the chemical composition of plantain and pasture. Plantain contained greater concentrations of organic matter, pectin, lignin ($P < 0.001$) and hot water soluble carbohydrate ($P < 0.01$) and lower concentrations of crude protein (CP), cellulose and hemicellulose compared with pasture ($P < 0.001$). Consequently the ratio of readily fermentable to structural carbohydrate was higher for plantain compared with pasture ($P<0.001$). Despite attempts via agronomic management to maintain herbage quality between periods, it was found that all

chemical components, except for pectin, of the feed offered of both diets changed significantly between periods ($P < 0.03$). Although, significant changes in chemical composition of pasture were found, the changes in pasture were relatively small compared with in plantain. Changes in chemical composition reflect the rapid growth and development of these forages during the mid spring season.

TABLE 1: Mean chemical composition (%DM) of perennial ryegrass-based pasture and plantain offered to deer during each period.

	Pasture		Plantain		Pooled
Period	1	2	1	2	SEM
Organic matter	88.1	89.5	91.1	92.8	0.29
ADF[2]	23.2	25.1	29.6	22.5	0.27
NDF[2]	41.0	43.9	43.1	32.9	0.07
CP[2]	24.4	19.0	12.7	11.5	0.08
HWSC[2] *(a)*	7.66	9.63	14.4	22.3	0.126
Pectin *(a)*	1.27	1.61	4.1	3.5	0.041
Hemicellulose *(b)*	17.8	18.8	13.5	10.4	0.27
Cellulose *(b)*	21.9	23.2	22.4	14.9	0.26
RFC:SC[1] *(a/b)*	0.22	0.27	0.51	1.02	0.03
Lignin	1.24	1.99	7.2	7.62	0.123
Gross energy (MJ /kg DM)	19.2	18.7	18.5	18.5	0.03

1Ratio of readily fermentable carbohydrates (a) to structural carbohydrates (b). 2ADF, acid detergent fibre; NDF, neutral detergent fibre; CP crude protein; HWSC, hot water-soluble carbohydrates.

The botanical composition of feed offered and refused is shown in Table 2. Plantain offered was found to consist of approximately 48 ± 11.3% DM (mean ± SD) of reproductive stem material which was selected against by deer so that approximately 66 ± 13.3 % DM (mean ± SD) of plantain refused consisted of stem. However, in contrast pasture was in a highly vegetative state, with no stem material present and there was little evidence of diet selection by deer fed pasture.

Dry matter intake of both forages by deer in both periods was similar at 1.57 and 1.67 ±0.101 SEM kg DM per day of pasture and plantain ($P > 0.5$), respectively.

TABLE 2: Mean botanical composition (%DM) of perennial ryegrass-based pasture and plantain feed offered and refused to deer.

	Botanical composition*				
	Leaf	**Stem**	**Clover**	**Weed**	**Dead**
Pasture					
Offered	95 ±2.1	0	2 ±1.4	0	3 ±0.7
Refused	82 ±19.0	0	4 ±2.7	<1	14 ±18.2
Plantain					
Offered	45 ±17.0	48 ±11.3	<1	2 ±1.4	5 ±3.5
Refused	26 ±11.3	66 ±13.3	<1	1 ±1.2	7 ±3.7

*Standard deviation of the mean is presented

As shown in Table 3, apparent digestibility of the chemical constituents of plantain were less than that of pasture ($P < 0.01$), except for the hot water soluble carbohydrate fraction and pectin which were almost completely digested. There was a significant main effect of period and a period by forage interaction for DM, OM, ADF, NDF and hemicellulose digestibilities which increased significantly from period 1 to period 2 for plantain ($P < 0.05$), but not pasture ($P > 0.1$). In contrast the digestibility of cellulose decreased between periods for plantain ($P < 0.01$).

TABLE 3: Mean apparent digestibility (%DM) of fresh perennial ryegrass-based pasture and plantain fed to red deer in each period.

	Pasture		Plantain		
Period	1	2	1	2	SEM[2]
Dry Matter	81.8	79.0	68.1	73.7	1.32
Organic Matter	83.4	79.5	72.2	77.6	1.15
ADF[1]	80.3	76.2	53.3	61.6	1.83
NDF[1]	82.8	79.8	50.6	62.8	1.11
HWSC[1]	95.0	97.7	97.1	97.5	0.32
Pectin	98.2	98.4	96.6	97.1	0.34
Hemicellulose	85.9	84.7	49.7	65.4	1.05
Cellulose	87.1	88.4	85.8	71.4	1.10
Gross Energy	81.6	78.5	69.3	73.0	1.32

[1]ADF acid detergent fibre; NDF neutral detergent fibre; HWSC hot water-soluble carbohydrates. [2]Pooled SEM.

The concentration of VFAs and ammonia in the rumen fluid of deer are shown in Table 4. The ammonia concentration in the rumen fluid was nearly five-fold less than when deer were fed plantain compared with pasture ($P < 0.01$). There was a trend for total VFA (mmol/L) in the rumen fluid of deer to be less when fed plantain compared with when fed pasture ($P < 0.09$). The molar proportions of acetic acid, iso-butyric acid, iso-valeric acid and the ratio of acetic: propionic acid were found to be less when deer were fed plantain compared with pasture ($P < 0.05$). Overall there was no effect of period or a significant interaction of period by forage for ammonia and most of the VFA's.

TABLE 4: Mean (SEM) concentrations of fermentation products in the rumen fluid of deer fed fresh perennial ryegrass-based pasture or plantain.

	Pasture	**Plantain**	**SEM**
Total VFA concentration (mmol/L)	68.5	57.8	4.28
VFA molar proportion (%)			
Acetic	61.6	57.3	1.06
Propionic	22.1	23.1	0.52
n- butyric	10.0	16.0	1.08
Iso -butyric	2.2	1.0	0.09
Iso-valeric	2.7	0.8	0.13
n-valeric	1.5	1.6	0.14
Acetic:Propionic ratio	2.81	2.48	0.090
Ammonia (mmol/L)	20.9	4.6	2.26

DISCUSSION

Forage feeding value can be defined as the animal production response to forage grazing under unrestricted conditions and is a function of both VFI and nutritive value. Nutritive value is dependent on the nutrient composition, availability and associated digestive processes. i.e. apparent digestibility (Ulyatt *et al.,* 1980; Waghorn & Clark, 2004). Plantain is a novel herb that appears suitable for deer production, yet there is limited information regarding the feeding value of plantain for deer and other ruminants. Hoskin *et al.* (2005) found pasture and plantain grazed in spring from October to December by weaner deer appeared to have a similar feeding value as determined by liveweight gain. However, in the following autumn from March to May, on the same swards, the liveweight gain of deer was 14% higher when grazing plantain compared with pasture (Hoskin *et al.,* 2005). Similarly, lambs grazed on plantain or pasture from December to March, under irrigated conditions, grew 39% faster when fed plantain (Moorhead *et al.,* 2002). Reasons for the apparent seasonal variation in feeding value of plantain compared with pasture needs to be elucidated, but are probably most likely due to changes in either VFI or apparent digestibility and may be associated with the propensity for plantain to rapidly 'bolt' into reproductive development during spring (Sanderson *et al*., 2003).

It is believed that this study is the first to determine the *in vivo* apparent digestibility of forage plantain by ruminant livestock. The apparent OM digestibility of both forages in the present study was found to be high. This was particularly so for pasture (81.5%), with OM digestibility of spring plantain in the current study (74.9%) more similar to previous studies with comparable deer fed the same ryegrass pasture-based sward as used in this study during autumn (72.3%, Hoskin *et al.,* 1995) and summer (74.4%, Kusmartono *et al.,* 1997).

The inferior digestibility of plantain compared with pasture in the current study is in conflict with the findings of Burke *et al.* (2000) and Hoskin *et al.* (2005). In both cases the effective DM degradability (82.6%) Burke *et al.* (2000) or in vitro apparent digestibility of OM (74.2%) Hoskin *et al.* (2005) of plantain was found to be greater than that of perennial ryegrass-based pasture (76.2% and 64.1%, respectively). However, the forages used in studies by Burke *et al.* (2000) and Hoskin *et al.,* (2005) were strictly vegetative and harvested in autumn, and not partially reproductive, or harvested in spring, as in the current study. Stewart (1996) suggested that the apparent digestibility of plantain, when harvested in spring and with the stem component removed, was not dissimilar from pasture when assessed by *in vitro* techniques. However, without information on factors influencing chemical composition and hence digestibility of forages, such as maturity, botanical composition and age of sward, it is difficult to accurately compare these various studies. Nonetheless, it appears that apparent digestibility and feeding value of plantain may not necessarily be greater compared with perennial ryegrass, but this may depend on a number of factors, including season. This is in contrast to comparisons between the other commercially available forage herb, chicory (*Cichorium intybus*), which always appears to have a higher feeding value and apparent digestibility compared with pasture when both forages are in the vegetative state (Barry, 1998).

The markedly reduced ammonia concentration in the rumen fluid of deer fed plantain suggests the possible presence of plant secondary compounds, such as condensed tannins, which inhibit rumen microbial proteolytic activity (Min *et al.,* 2002). Wang *et al.* (1996) showed that when sheep were fed a legume (*lotus corniculatus*) containing high concentrations of total condensed tannins (39.5g/kg DM) the ammonia concentration within the rumen fluid was reduced by 49% compared with ewes fed the same forage, but additionally supplemented with polyethylene glycol (PEG) to neutralise the effect of the condensed tannins. Nutritionally this could be beneficial to ruminants as it reduces the amount of protein wasted when excess ammonia is absorbed from the rumen and excreted as urea and can result in an increased availability of essential amino acids and hence a greater efficiency of use of dietary protein (Hoskin *et al.,* 2003).

Burke *et al.,* (2000) suggested the presence of secondary plant compounds in plantain may have contributed to a rapid digestion *in sacco*, but minimal digestion *in vitro,* as *in vitro* digestion was considerably slower than in sacco degradation. Plantain has been reported to contain phenylethaniod glucoside and iridoid glycoside compounds that have potential bioactive effects within the gastrointestinal tract of animals (Tamura & Nishibe, 2002). Condensed tannins present in members of the Plantaginaceae genus have received much interest, however, the concentration of condensed tannins in the plantain cultivar (Ceres Tonic) used in this study was not measured. Previous attempts to determine the condensed tannin content of the swards used in the present study (Swainson, 2004) found that the concentration of total condensed tannins in plantain

(0.20 % DM) were minimal and apparently similar to pasture (0.17 % DM). However, common tests used to detect condensed tannins may not be suitable for plantain. Stewart (1996) reported concentrations of condensed tannins in Ceres Tonic plantain of between 0.4 and 1% with both the vanillin-HCL and butanol-HCL techniques (Jackson *et al.,* 1996). However resultant extracts presented abnormal colours under both tests and there was an unusually slow response to the vanillin-HCL test. Therefore, further research is needed to determine the secondary compound content, including condensed tannins, of the new forage plantain cultivar Ceres Tonic.

Reduced rumen ammonia concentrations can simply be due to a lower crude protein content of the diet, as observed for plantain compared with pasture in the current study. However, the difference in crude protein content of the forages was 2-fold, whereas the difference in rumen ammonia concentrations was 5-fold. Additionally, the volatile fatty acids iso-butyric and iso-valeric which are products of branched chain amino acid fermentation (Van Soest, 1994; Wang *et al.,* 1994) were found to be much lower when animals were fed pasture. Wang *et al.* (1994) and Wang *et al.* (1996) observed similar differences in VFA proportions of iso-butyric and iso-valeric acids when sheep were fed *Lotus corniculatus,* and some of the sheep were supplemented with PEG. Ideally further work should be conducted to confirm the present findings of decreased ammonia concentrations in the rumen fluid of deer fed plantain compared with pasture, but when both forages have the same crude protein concentrations, and/or any potential effect of condensed tannins is neutralised. However, as it is unlikely that protein levels in both forages would be similar for a sufficient period of time to allow a further comparison, a similar digestibility trial to the one presented here should be conducted with half the animals on each diet supplemented with PEG.

In conclusion plantain (Ceres Tonic) appears to have a lower apparent digestibility, yet similar feeding value compared with perennial ryegrass-based pasture in spring. However this may not be the case in summer and autumn and further in vivo digestibility trials and grazing studies, including measurement of VFI, are required for a full understanding of the factors affecting the feeding value of plantain. It appears that plantain may improve the efficiency of digestion of protein in deer. However, it remains uncertain if secondary compounds are responsible for these changes in protein fermentation in the rumen. Investigation of the effects of plantain (Ceres Tonic) and its secondary compounds, on the rumen microbial ecosystem is therefore warranted.

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