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DEEResearch Postgraduate Fund

Project Title: Forage herbs for natural minerals – a comparison of perennial ryegrass-based pasture, chicory and plantain.

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Background:

Chicory produces a large quantity of high nutritive value herbage from spring through to autumn. Chicory has proven to be of high feeding value for deer in several trials and is highly suited to deer production systems because the annual pattern of feed production is aligned with deer feed requirements.

Of the farmed ruminant species in New Zealand, farmed deer seem particularly sensitive to mineral deficiencies and this could be related to the restricted access of farmed deer to plant dietary constituents rich in minerals such as herbs and browse, which deer in the natural state readily consume. In particular, copper deficiency, and in key locations cobalt, selenium and iodine deficiency are of increasing concern to the deer industry. Widespread intervention using copper needles or boluses has been reported (P.R.Wilson, pers comm.).

Chicory contains a high mineral content relative to other conventional forages. In two trials at Massey University deer grazing chicory have been shown to have an improved copper status compared with deer grazing perennial-ryegrass based pasture for whom a significant proportion were copper deficient (Barry *et al*., 2001; Wilson *et al*., 2003). However, it is unknown whether improving the mineral status of deer will also increase venison mineral concentrations and that could potentially impact on human health and wellbeing.

Plantain is a new forage herb that is claimed to be suitable for deer production due to a similar annual pattern of feed production to chicory, a high mineral content and a claimed greater plant persistence under grazing than chicory. However no published data exists on the feeding value of plantain for farmed deer and no direct comparisons of the two forage herbs, chicory and plantain, have been made for farmed ruminants in New Zealand.

Project Aims:

1. To determine the seasonal changes in mineral content of perennial-ryegrass based pasture, chicory and plantain.
2. To compare the mineral status of young deer grazing ryegrass-based pasture, chicory or plantain.
3. To initiate investigation into the effect of grazing forage herbs on the mineral content of venison.

Methods

Aim 1:

Bi-monthly forage samples (400g fresh weight) cut to ground level were taken from chicory and plantain paddocks described below from March 2003 to March 2004, pooled bi-monthly per forage, washed and stored at -20ºC for mineral content determination using Plasma Emission Spectrometry (Spectrachem). Repeated measures analysis (SAS) was used to determine effects of time (month) and forage on herbage mineral content.

Aims 2 & 3:

On 30 September 2003, 30 weaner deer on the Massey University Deer Research Unit were allocated to grazing either permanent perennial ryegrass (*Lolium perenne* cv Nui)- based pasture, chicory (*Cichorium intybus* cv Grasslands Puna) sown spring 2000 or narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) sown spring 2002 (Plate 1). There were 10 weaners (7 pure red stags and 3 ¼ wapiti hinds) per forage treatment, allocated on the basis of sex and live weight and at the start of the trial all deer used had been grazing ryegrass-based pasture together in one mob for 3 months. All deer were treated with anthelmintic (moxidectin "Cydectin" pour-on, Fort Dodge Animal Health) at the start of the trial. Pasture comprised 4 paddocks (1.65ha in total), chicory comprised 3 paddocks (1.21ha in total) and plantain comprised 3 paddocks (1.9ha in total).

Deer were rotationally grazed throughout the trial with herbage allowances (Semiadi *et al*., 1993) (excluding dead, reproductive stem and weed material) for all animals being set at 7kg dry matter per deer per day. Rotation length was 3 weeks for chicory, 4 weeks for plantain and for pasture, deer did not return to paddocks previously grazed within the timeframe due to dropping paddocks out for calving.

Pre- and post- grazing herbage masses were determined from each paddock, together with sampling for feed offered and botanical composition by taking cuts to soil level from 6 quadrats ($0.1m^2$) per paddock. Samples of feed on offer were mixed and two 200g subsamples taken for pooling per forage and storage at -20ºC for chemical and mineral analysis. Samples for botanical composition were dissected into grasses, legume, dead matter and weed (pasture), and leaf separately to stem, legume, dead matter and weed (chicory and plantain). Each component was separately oven-dried (100ºC for 18h) and weighed. Hand-plucked samples estimating deer diet selected (Kusmartono *et al*., 1996) were taken daily and pooled per paddock at -20ºC for chemical analysis (Hoskin et al., 1999) and mineral content (Plasma Emission Spectrometry, AgResearch Ltd).

Deer were weighed on a fortnightly basis to determine liveweight gain. At the start of the trial, liver biopsies were taken from sedated deer using local anaesthetic for copper status determination (Wilson, 2000). At the trial conclusion on 1st December, liver samples were recovered for copper status determination (Gribbles Veterinary Pathology) following slaughter at Venison Packers Feilding Ltd. Blood samples were taken by jugular venipuncture at the start, mid point (4 weeks) and end of the trial for selenium and serum vitamin B12 concentration determination (Gribbles Veterinary Pathology). Tissue samples (100g) from the muscles at the top of the neck (upper trapezius and sternocleidomastoid) discarded following removal of the head from the carcass were taken, sinew and silverskin removed and freeze dried for later mineral content determination using Plasma Emission Spectrometry (Spectrachem). Carcass weight data was obtained from Venison Packers Feilding. Velvet length (mean of both sticks per animal) and total weight per animal was obtained from spikers that required velvetting before transport to slaughter.

Final liveweight, average liveweight gain, carcass weight, and velvet data were analysed using the glm procedure ANOVA in SAS (SAS, 1998). Mineral data was analysed using the mixed model procedure in SAS (SAS, 1998). Values reported are least square means (LSM), with standard error of the mean (SEM). 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 of treatment was detected.

Plate 1. Perennial ryegrass-based pasture (left), chicory (middle) and plantain (right).

Results and discussion:

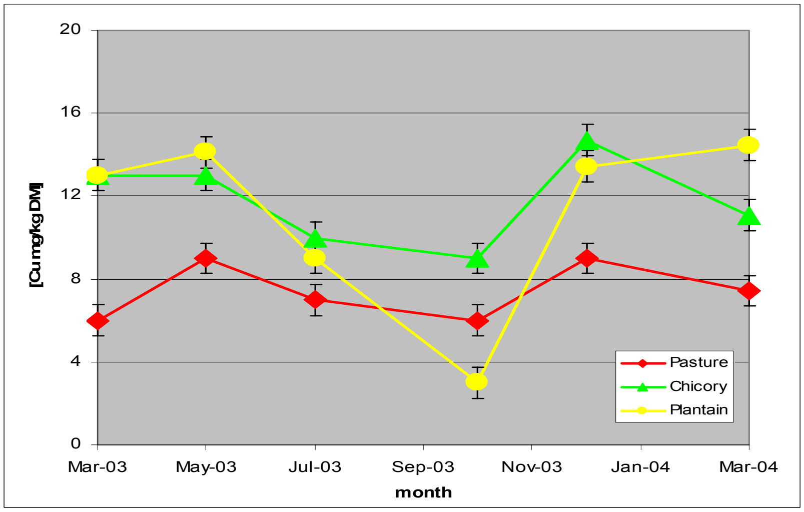
Aim 1:
Differences in mineral content of forages for zinc, copper, titanium, potassium, chlorine, sulphur, silicon, aluminium and sodium were found (P<0.05). Significant seasonal variations (P<0.05) were found for zinc, copper, iron, calcium, potassium, sulphur, silicon and sodium, with effects for copper, zinc and calcium shown in Figures 1a,b,c, respectively. For most of the macro- and micro-minerals, specific requirements for deer have not been determined. Due to concern about copper deficiency in the deer industry, the discussion of this large data set will be limited to the results obtained for copper. However, data for other minerals can be obtained from S.O.Hoskin@massey.ac.nz.

Grace *et al*. (2003) suggested that 11mg/kgDM copper may be an adequate dietary level for deer, provided interactions with other minerals such as molybdenum do not reduce absorption. In looking at Figure 1a, it can be seen that copper concentrations in all forages fall to low levels during winter to late spring and all are below the recommended dietary minimum during this period. In late spring, copper concentrations in the forage herbs rose rapidly to above the recommended dietary minimum level. On the other hand, despite a rise in late spring, the copper concentration of ryegrass-based pasture remained below the recommended minimal level.

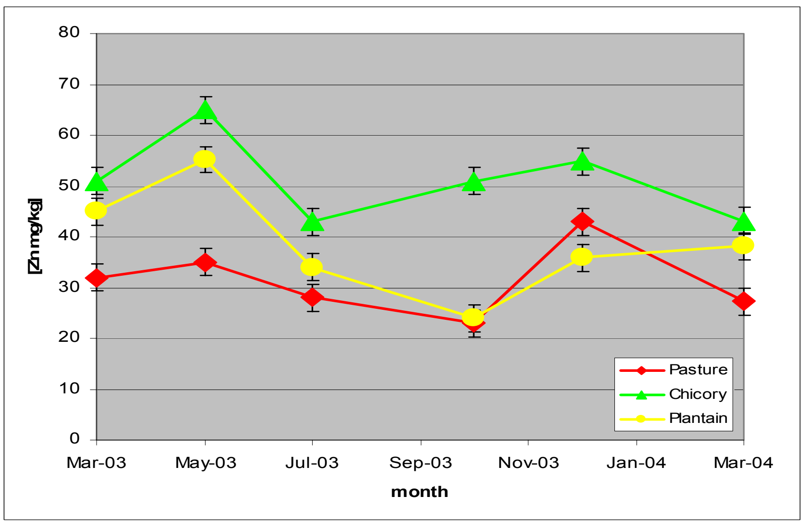
The mean copper concentration over the year was 7.2, 12.3 and 11.3mg/kgDM (±0.75 SEM) for pasture, chicory and plantain, respectively, with concentrations in pasture significantly lower than for both forage herbs (P<0.001). At no time was the copper concentration of pasture above the recommended dietary minimum. Of the weaner deer grazing on this pasture from birth to 10-11 months old (used in second part of this report), only 20% had an adequate liver copper status, 40% had a marginally deficient copper status and 40% were classified as copper deficient and at risk of clinical copper deficiency on 1st October. No clinical signs of copper deficiency were observed. However, given the deficient copper status of these animals it is possible that poor copper status may have contributed to the low growth rates of weaner deer grazing perennial ryegrass-based pastures compared with deer grazing chicory in subsequent work (below) and in related trials by Barry *et al*. (2001) and Wilson *et al*. (2003).

There is conflicting evidence in the literature regarding the effect of copper supplementation (copper needles or injection) on deer growth, but then supplementation of animals that have an adequate copper status is unlikely to give a growth rate response. The advantages of forage herbs for mineral supply can include; a "natural", holistic and minimal intervention system which supports marketing initiatives (Loza, 2001), provision of a highly palatable forage of feeding value greater than conventional ryegrass-based pasture, greater bioavailability of minerals, potential for reduction of internal parasites due to nutrition, secondary compounds and plant physical characteristics and even possibly reduced methane production (Swainson *et al.,* 2004).

Figure 1a,b,c: Seasonal variation in the a (copper), b (zinc), c (calcium) concentration of pasture, chicory and plantain feed offered[1] to weaner deer at Massey University. [1]Cut to ground level



a



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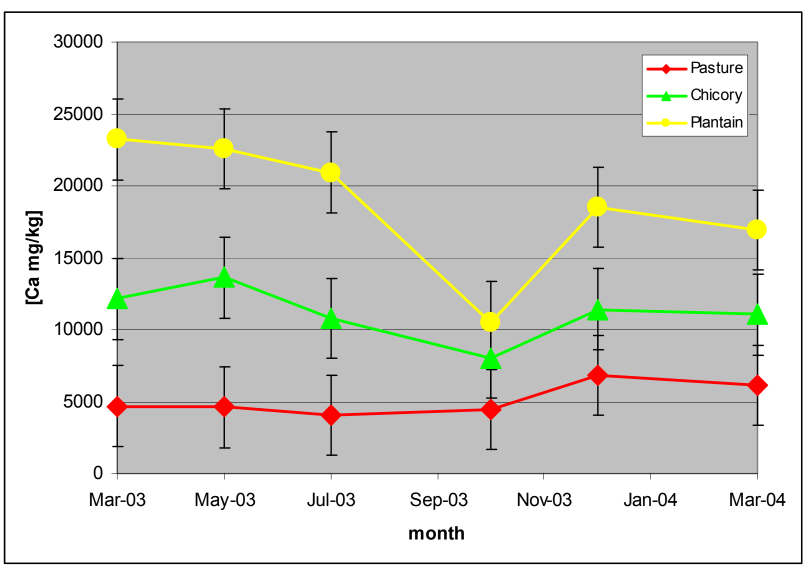

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Aims 2 & 3:

<u>Forages:</u>

Table 1a. Mineral composition (mg/kg freeze dried herbage) of minerals in ryegrass-based pasture, chicory and plantain total feed offered[1] and estimated feed selected[2] by grazing weaner deer from 29 September to 28 November 2003.

	Pasture	Chicory	Plantain
		Total Feed Offered	
Boron	5.8	30.4	26.7
Calcium	4,252	8,017	12,971
Cobalt	0.19	0.14	0.28
Chromium	0.80	0.4	0.52
Copper	6.1	8.4	6.3
Iron	499	270	407
Potassium	23,429	26,739	14,222
Magnesium	1,626	2,517	1,189
Manganese	199	110	123
Molybdenum	0.48	0.20	0.18
Sodium	2,443	3,033	4,891
Sulphur	1,726	1,797	1,557
Selenium[3]	<2	<2	<2
		Feed Selected	
Boron	57.6	91.4	84.3
Calcium	16,567	23,638	37,447
Cobalt	0.39	0.47	0.97
Chromium	2.4	1.66	1.39
Copper	28.7	34.3	21.1
Iron	1479	1175	526
Potassium	101,828	80,154	42,647
Magnesium	6,682	7,477	3,168
Manganese	517	353	499
Molybdenum	2.56	0.42	0.74
Sodium	6,281	13,335	16,045
Sulphur	8,948	6,802	5,756
Selenium[3]	<2	<2	<2

[1]Cut to ground level; [2]Hand plucked using the method of Kusmartono *et al*. (1996); [3]Se content below level of sensitivity of method of analysis.

Table 1b. Chemical composition of ryegrass-based pasture, chicory and plantain total feed offered[1] and estimated feed selected[2] by grazing weaner deer from 29 September to 28 November 2003.

	Pasture	Chicory	Plantain
		Total Feed Offered[1]	
Organic matter%	79	77	77
Nitrogen %	2.4	2.8	1.7
Gross energy kJ/g	16.1	15.9	15.5
		Feed Selected[2]	
Organic matter%	79	76	79
Nitrogen %	3.1	3.3	2.1
Gross energy kJ/g	16.7	15.8	16.1

[1]Cut to ground level; [2]Hand plucked using the method of Kusmartono *et al*. (1996)

Table 1a shows the mineral content of both feed offered and estimated diet selected of the treatment forages. In general, the mineral concentration of the estimated diet selected was higher than for feed offered. For example the content of copper in the estimated diet selected was 3.3-4.7 times higher than for feed offered and for cobalt this difference was 2-3.5 fold. This is likely to be due to animals selecting against dead matter and plant stem proportions in the sward, components that have a low concentration of trace minerals in particular. For estimated diet selected, pasture was highest in chromium, iron, potassium, manganese, molybdenum and sulphur content; plantain was highest in calcium, cobalt and sodium and chicory was highest in boron, copper and magnesium content. Feed offered followed the same trends except for sulphur and potassium which were highest in chicory and not pasture.

Table 1b shows the organic matter, nitrogen and gross energy content of the treatment forages determined by wet chemistry. Organic matter and gross energy content of feed offered and selected of all forages was similar. The nitrogen content of forages increased from feed offered to diet selected as deer tend to select the immature plant parts that are high in nitrogen. The nitrogen content of plantain is lower compared with both pasture and chicory.

Botanical composition of the forages (not presented) was for pasture - 91% grass, 2% clover, 7% dead matter and 1% weed; for chicory - 33% chicory leaf, 5% chicory reproductive stem, 28% clover, 12% dead matter and 22% weed (mostly pennyroyal and dock), for plantain – 46% plantain leaf, 25% plantain reproductive stem, 2% clover, 23% dead matter and 4% weed (mostly thistles).

Animal Mineral Status

Copper:

Table 2 shows both the liver copper concentration and the proportion of each group with inadequate copper status and therefore “at risk” of clinical copper deficiency at the initiation and conclusion of the trial. Initial liver copper status of the treatment groups was similar. However, liver copper concentrations at slaughter from deer grazing pasture was significantly lower compared with from deer grazing chicory. Liver copper concentrations of deer grazing plantain were intermediary between pasture and chicory and did not differ significantly from either. Over the 8 week trial liver copper status of the pasture group declined by 17%, and the liver coper status of the chicory and plantain groups increased by 336 and 184%, respectively. Based on liver copper concentrations at the conclusion of the trial, 90% of deer on pasture, 0% of deer on chicory and 30% of deer on plantain were copper deficient and “at risk” of clinical copper deficiency. This is despite the copper concentrations of the estimated feed selected of all forages apparently being above the 11mg/kgDM suggested by Grace *et al*. (2003) as being the minimum dietary copper requirement for deer in the absence of high concentrations of elements such as iron, molybdenum and sulphur which may decrease absorption of copper. It is not known whether the higher concentration of molybdenum and iron in pasture compared with the forage herbs was enough to have reduced copper absorption in this group.

Table 2. Weaner deer copper status.

	Pasture	Chicory	Plantain	SEM	Effect of Forage[1]
	Liver Copper Concentration (μmol/kg)				
Initial	97	89	90	19.3	NS
Final	81[a]	299[b]	166[ab]	42.0	*
	% At risk of clinical copper deficiency[2]				
Initial	60	30	30		
Final	90	0	30		

[a,b] Differences between letters within rows indicate significant differences (P<0.05) between treatment groups (P<0.05).
[1]*(P<0.05), **(P<0.01).
[2]Based on liver copper concentration <60μmol/kg, no statistics performed.

Vitamin B12:

Table 3 shows 4-weekly serum vitamin B12 concentrations. Vitamin B12 status of ruminants is related to the cobalt content of the diet, but no reference values or relationships between dietary cobalt intake and animal requirements have been established for deer (Grace and Wilson, 2002). There was no difference in serum vitamin B12 status between forage groups at the start of the trial. At the start, 80% of deer on pasture, 90% of deer on chicory and 60% of deer on plantain had low vitamin B12 status (sheep reference values), or vitamin B12 concentrations were below the detectable level (<57 pmol/L) as determined by the Gribbles Veterinary Pathology Laboratory. However, in all forage groups the serum vitamin B12 status significantly increased during the first 4 weeks to the trial midpoint (P<0.01). After only four weeks, the serum vitamin B12 status of pasture and chicory groups was significantly lower compared with the plantain group (P<0.01). From the mid point onwards the serum vitamin B12 concentration subsequently declined in all groups. At the trial conclusion, the serum vitamin B12 status of deer grazing plantain was significantly greater than at the trial initiation and compared with deer grazing the other forages. At the trial conclusion 90% of deer on pasture, 60% of deer on chicory and 10% of deer on plantain had low vitamin B12 status according to reference values established for sheep.

Table 3. Weaner deer vitamin B12 status.

	Pasture	Chicory	Plantain	SEM	Effect of Forage[1]
	Serum B12 Concentration (pmol/L)				
Initial	83	98	118	29.4	NS
Mid point	142[a]	183[a]	389[b]	32.4	**
Final	101[a]	148[a]	246[b]	31.7	**

[a,b] Differences between letters within rows indicate significant differences (P<0.05) between treatment groups (P<0.05).
[1]*(P<0.05), **(P<0.01).

Selenium:

Table 4 shows the mean group blood selenium concentration at the trial start, mid point and end. All animals appreared to have adequate selenium status at all times. This was despite all forages having selenium concentrations below 0.2mg/kgDM which is thought to be inadequate for sheep. Selenium status of the treatment groups at the start was similar. At the trial midpoint, the selenium status of deer on chicory was significantly lower compared with deer on plantain, with deer on pasture intermediate and not significantly different from those on either herb. At the conclusion of the trial, blood selenium status of deer grazing both chicory and pasture was significantly lower compared with deer grazing plantain.

Table 4. Weaner deer blood selenium status.

	Pasture	Chicory	Plantain	SEM	Effect of Forage[1]
	Blood Selenium Concentration (nmol/L)				
Initial	295	252	328	18.2	NS
Mid point	285[ab]	239[a]	341[b]	15.8	*
Final	279[a]	205[a]	377[b]	20.1	*

[a,b] Differences between letters within rows indicate significant differences (P<0.05) between treatment groups (P<0.05).
[1]*(P<0.05), **(P<0.01).

Venison Mineral Content

Table 5 shows that for this preliminary investigation there were no differences between forage species grazed by deer on the mineral content of venison. The method used was not able to detect all minerals present in venison, particularly those at low concentrations such as copper and selenium, and this warrants further investigation. Although the total iron content of venison from deer grazing the three forages was similar, further work is being conducted to determine whether there is any difference in the relative bioavailability of iron.

Table 5. Mineral content of venison (mg/kg freeze dried muscle)[1]

		Forage			Effect of
	Pasture	Chicory	Plantain	SEM	Forage
Sodium	4,035	3,839	3,778	141.0	NS
Phosphorus	8,653	8,594	8,612	91.3	NS
Sulphur	8,318	8,461	8,331	84.9	NS
Silicon	86.4	36.2	51.4	18.7	NS
Zinc	250	248	241	5.15	NS
Calcium	249	257	245	12.03	NS
Iron	116	114	118	4.13	NS
Potassium	13,881	13,834	14,059	142.8	NS
Magnesium	570	560	577	19.5	NS

[1] upper trapezius and sternocleidomastoid muscles

Animal Production

Comparative feeding value of forages is determined by measuring the production response of animals grazing replicated plots or paddocks of the test forages at the same herbage allowance over a period of a minimum of 6 weeks and typical measurements of feeding value include liveweight gain, milk production etc. Table 6 shows there was a significant effect of forage grazed on average liveweight gain, final live weight and carcass weight, but not velvet production.

Feeding value of chicory in spring was approximately 45% greater than for ryegrass-based pasture or plantain, which had similar feeding value. After 8 weeks of grazing, this difference in feeding value resulted in the mean carcass weight of chicory-grazed deer being on average 10% heavier than for pasture or plantain-grazed deer. It is not known the degree to which differences in mineral status between treatment groups may have influenced deer growth rate.

Six stags on each of both pasture and chicory and 7 stags on plantain were velvetted. Due to the low number of animals and the high variability associated with the velvet production data, no statistically significant effect of forage was observed for velvet production. However, it is interesting to note in this preliminary investigation that the average length of spiker velvet sticks produced from herb-grazed animals was twice that of pasture-grazed animals and the weight of velvet harvested from animals grazing the herbs was 40-70% greater than from animals grazing pasture. This could warrant further investigation.

Table 6. Liveweight gain (LWG), final liveweight (LW), carcass weight (CW) and spiker velvet production.

	Pasture	Chicory	Plantain	SEM	Effect of Forage[4]
		Venison Production			
LWG (g/d)	217[a]	303[b]	204[a]	19.0	**
Final LW (kg)[1]	86[a]	91[b]	85[a]	1.34	*
CW (kg)	47.6[a]	51.3[b]	45.2[a]	1.40	*
		Spiker Velvet Production			
Velvet length (cm)[2]	10	23	26	6.9	NS
Velvet weight (g)[3]	121	205	176	63.7	NS

[a,b] Differences between letters within rows indicate significant differences (P<0.05) between treatment groups (P<0.05). [1]Final live weight adjusted for initial live weight. [2]Group average calculated using average of both sticks per animal. [3]Group average calculated using total of both sticks per animal. [4]*(P<0.05), **(P<0.01).

The main differences in botanical composition between pasture and the herbs (above) was the high proportion of weed present in the herb swards and for chicory, the high clover content. Plantain had a higher stem and dead matter content and lower clover content compared with chicory. This meant that for plantain, deer were exposed to a sward containing 52% of relatively unpalatable herbage components compared with values of 8% within pasture and 39% within chicory swards. Although herbage allowances were adjusted accordingly, deer grazing plantain would have had more difficulty in selecting the more palatable components of the sward. For forage herbs in general, as the reproductive stem and dead matter content increases the feeding value decreases. Therefore control of plantain swards in late spring by mechanical topping or grazing with other stock following weaner deer is likely to be important in maintaining feeding value and in this trial if reproductive stem development had been better controlled the growth of weaner deer grazing plantain may have been greater.

Another of the many factors which may have influenced feeding value of the swards evaluated was the legume content, which was 14 times higher for chicory than for pasture and plantain. Although the chicory was sown as a pure crop, volunteer white clover had established since and it is possible that if the plantain sward had had a similar legume content the relative feeding value recorded in this study for plantain may have been different.

Summary and conclusions

1. These results suggest a potential role for forage herbs in naturally maintaining adequate trace element status of deer. However due to influences of soil chemistry and pH on trace mineral status of plants, similar studies need to be conducted in a range of environments before data can be extrapolated from the present trial to other environments.
2. Based on this preliminary study it appears that chicory and plantain may have complementary roles in mineral provision as chicory boosted copper status and plantain boosted vitamin B12 and selenium status and use of swards containing both forage herbs may provide a better balance of dietary minerals than single-herb swards.
3. Feeding value of chicory in spring was approximately 45% greater than for ryegrass-based pasture or plantain, which had similar feeding value - leading to a carcass weight advantage of 10% for deer grazing chicory for only 8 weeks prior to slaughter.
4. This study has not shown any effect of diet on venison mineral content, but this work is continuing.
5. Establishment of reference values for selenium and vitamin B12 status of deer is required, in addition to knowledge of relationships between dietary cobalt and selenium, and deer requirements.

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