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Use of a micro-sward technique for determining bite mass of four grass species in short-term tests

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Abstract Four grass species typically found in semi-natural pastures in the UK were sown individually in boxes (85 × 44 × 14 cm) to create micro-swards. Yorkshire fog (*Holcus lanatus* L.); creeping bent (*Agrostis stolonifera* L. cv Kromi); rough meadow-grass (*Poa trivialis* L.); and perennial ryegrass (*Lolium perenne* L. cv Glen) were offered to yearling Simmental × Holstein steers in short-term tests to characterize bite mass in relation to morphological traits of the grass species. Boxes were weighed (±0.1 g) before and after a 5 min period during which the steers were allowed to take ~50 bites. Perennial ryegrass had the greatest sward surface height (17.7 cm vs. mean 12.7 cm for the other species). Extended tiller length was greatest for ryegrass (17.3 cm) and least for rough meadow-grass (13.5 cm). Leaf length was greatest for ryegrass (6.5 cm) and Yorkshire fog (7.0 cm) and least for creeping bent (4.0 cm). Sward DM% was lowest for ryegrass (19.7%) and highest for creeping bent and rough meadow-grass (mean 23.2%). Number of bites taken from the boxes did not differ between grass species (mean 32.7 bites). Mean dry matter bite mass was greatest for perennial ryegrass (0.50 vs. mean 0.37 g DM bite^{-1} for the other species). Regression analyses indicated that the best sward variables for predicting mean DM bite mass were sward DM% (F prob. = 0.066) and sward surface height (F prob. = 0.086). While morphological characteristics affected bite mass, more is yet to be learned about other factors that may affect intake characteristics of forage species to improve economic and environmental sustainability.

Keywords Bite mass · Cattle · Sward boxes · Grass species

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Introduction

Interest in complex multi-species plant communities typically found in semi-natural grasslands has increased globally in recent years. Changes in agricultural support schemes and other environmental legislation are putting direct pressure to conserve, enhance, or restore such grasslands for their intrinsic biodiversity value as a public good. Emphasis is being placed on managing multi-species grasslands within the context of sustainable farming systems compared to managing primarily for efficient and competitive livestock production. Increased input costs are driving dairy and beef

producers to seek lower-cost production methods such as managed grazing to enhance economic and environmental sustainability. Additionally, increased consumer awareness of health and environmental benefits of pasture-based meat and milk products is creating a rapidly expanding market, particularly in organic animal agriculture.

A number of studies have been conducted that evaluate the impact of grazing animals on these semi-natural swards (Grant and Hodgson 1986; Olff and Ritchie 1998; Bullock and Oates 2000) and conversely, of the effect of using biodiverse swards on the weight gain, milk production, efficiency and product quality of grazing animals (Soder et al. 2006). However, studies of the mechanisms by which these effects occur have to date been relatively crude. Most studies have only evaluated whole sward effects (e.g., Soder et al. 2006). Where they have gone further, dietary choice was only identified to the level of plant functional group (e.g., Dumont et al. 2007) and not at the plant species level. Indeed, how intake characteristics relate to plant characteristics is not generally known. Thus, an essential first step is to improve our understanding of how characteristics of the individual plant species in these mixed swards affects bite mass (Laca et al. 1994) of grazing animals. A considerable body of work has examined simple mixtures of perennial ryegrass and white clover where the individual intake characteristics of the species were first studied before proceeding to studies of dietary choice (e.g., Newman et al. 1992; Penning et al. 1995, 1997). Penning et al. (1991a) successfully utilized this approach in simple mixtures of perennial ryegrass and white clover.

Few field-scale experiments that evaluate intake characteristics have been conducted (Hazard et al. 1998; Barrett et al. 2003; Orr et al. 2003) as this type of research is labour-intensive and expensive. Orr et al. (2005a) developed a low-cost, short-term method of assessing bite mass of different perennial ryegrass cultivars and demonstrated that differences observed at the micro-sward box level were correlated with those found in the field (Orr et al. 2005b). While this methodology has been conducted and reported in the literature, further studies of this kind are justified to broaden the range of forage species studied. Specifically, the generality of drivers of grazing behavior across forage species needs to be tested. Therefore, the aim of this study was to characterize bite mass of cattle in relation to morphological traits of grass species typically found in semi-natural pastures in the UK following the methods of Orr et al. (2005a).

Materials and methods

Grass species

The experiment was conducted in 2005. The grass species chosen were native to the UK (Stace 1991) and were representative members of a typical mesotropic MG6 lowland semi-natural grassland community as defined in the UK National Vegetation Classification by Rodwell (1992). MG6 is the major permanent pasture type on moist but freely-draining circumneutral brown soils in lowland Britain. They had contrasting morphology and were creeping bent (*Agrostis stolonifera* L. cv Kromi), Yorkshire fog (*Holcus lanatus* L.), rough meadow-grass (*Poa trivialis* L.) and perennial ryegrass (*Lolium perenne* L.). Wild forms of Yorkshire fog and rough meadow grass were sown whereas a selected allotetraploid (Warnke 2003) cultivar of creeping bent (Kromi) was sown, reflecting seed availability. The perennial ryegrass cultivar (Glen) studied previously using these same methods by Orr et al. (2005a) was also included. Hubbard (1984) classified creeping bent as a tufted perennial, 8–40 cm high, spreading by leafy stolons and forming a close turf; Yorkshire fog as a loosely to compactly tufted softly hairy perennial, 20–100 cm high; rough meadow-grass as a loosely tufted perennial, 20–100 cm high, with creeping leafy stolons and perennial ryegrass as a loosely to densely tufted perennial, 10–90 cm high.

Micro-sward establishment

The four grass species were each sown separately (Table 1) in nine replicate plastic boxes (85 cm × 44 cm × 14 cm, weighing 3 kg when empty) per species (36 boxes total) on 1–4 February (nine boxes of one species each day) at the former Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon, UK (50°46′10″N, 3°54′05″W). All boxes had 6 mm drainage holes drilled in the base spaced at 11 cm in three rows of seven (21 holes total). A layer of capillary matting was placed on the bottom

Table 1 Micro-sward sowing, harvesting, and testing dates

	Creeping bent	Yorkshire fog	Rough meadow-grass	Perennial ryegrass
Sowing	1 February	2 February	3 February	4 February
Harvest 1	5 April (64 days)	–	–	5 April (60 days)
Harvest 2	19 April (14 days)	19 April (76 days)	19 April (75 days)	19 April (14 days)
Harvest 3	10 May (21 days)	10 May (21 days)	10 May (21 days)	10 May (21 days)
Harvest 4	31 May (21 days)	31 May (21 days)	31 May (21 days)	31 May (21 days)
Testing	21 June (21 days)	21 June (21 days)	21 June (21 days)	21 June (21 days)
Sowing-test	140 (days)	139 (days)	138 (days)	137 (days)

Four boxes, one for each variety, tested on each day

Note: Values in brackets indicate the number of days between sowing and the first harvest, between subsequent harvests and between the final harvest and testing

of each box which was then filled with a loam-based compost (John Innes No. 3; http://www.jic.bbsrc.ac.uk/) containing peat, sand and nutrients. The declared nutrient concentration ranges for the compost were for N (201–244 ppm), P (161–200 ppm) and K (321–360 ppm), respectively. A plastic mesh (50 mm × 30 mm openings) was placed within the compost, positioned 7 cm below the surface. This was intended to ensure that, once the roots had penetrated and enveloped the mesh, individual tillers were less likely to be pulled out by the cattle.

The seeds were sown at a rate of 8,000 seeds m^{-2}. Five seeds were sown in each cell of a temporary grid comprising of 25 mm × 25 mm squares placed on top of the compost surface with the aim of ensuring a uniform distribution of established plants across the box. Once watered and with the plants established, each box weighed ~50 kg.

Micro-sward management

The boxes were kept in a glasshouse from establishment until day of use and were hand-watered when the soil surface was deemed dry. The micro-swards were cut using scissors to 6 cm above the root-shoot interface as measured by a graduated vertical marker-guide on the dates shown in Table 1, i.e., at ~21 day intervals. Two boxes of each grass species were cut on four consecutive days (except the fourth day when 3 boxes were cut) beginning on the date noted in Table 1, in order to maintain a similar cutting interval for all four eventual test days. The objective of this management was to maintain the micro-swards in a vegetative state, encourage rapid tillering, and increase the sward density to simulate as closely as possible a well-established sward in field conditions.

By 5 April, it was deemed that the Yorkshire fog establishment was poor and in order to rectify this, plants were transplanted from the four worst Yorkshire fog boxes to the other five boxes. In addition, wild plants collected from a hedgerow were also transplanted to these five boxes and these were used for the tests. On 12 April, four boxes (those from which Yorkshire fog plants had been transplanted) were re-sown and these were used for training the test animals.

Following a final 21 day re-growth period, the micro-swards were moved out of the glass houses to the test arena on the day of each test.

Experimental design

Each grass species was offered to each of four steers in a 4 × 4 Latin-Square design with 4 periods (days; 21–24 June). The order in which the tests on each of the four species were conducted within any day was randomized.

Animals

Four yearling Simmental × Holstein steers were used for the micro-sward tests. The mean weights of the steers at the time of the test were 523 ± 22 kg. Steers grazed an MG6 grassland typical of the target community of interest from 9 May until 13 June when they were moved to a predominantly ryegrass pasture adjacent to the test arena. Animals remained in this area except for ~1 h prior to testing period each day in order to ensure they were not fasted before each test so

as not to affect intake (Greenwood and Demment 1988; Newman et al. 1994; Patterson et al. 1998).

Animal training

Training of animals commenced on 14 June and each steer was allowed to remain in a test arena (3 × 3 m) with a sward box for ~5 min or until they had taken 50 bites, whichever came first. The test arena was constructed using cattle hurdles in a covered building with a concrete floor. The steers were acclimated to the test arena for 4 days prior to the initiation of the experiment using the same protocol as during the experimental period. The boxes were weighed in a wind-proof enclosure (3 × 2.5 m wooden shed) from which the animals were observed and video-recorded during the tests. The micro-swards were placed on the floor of the arena so that the root-shoot interface was ~14 cm above the level at which the steers were standing.

Measurements

Immediately before each test, 24 sward surface height (SSH) measurements were taken on each micro-sward using a sward stick (Bircham 1981). Measurements were collected from three rows of eight randomly placed (within row) measurements per row with rows parallel to the long side of the micro-sward box and approximately equidistant from each other and the sides of the box.

Before each test, ten tillers were cut at the root/shoot interface from each micro-sward. Extended tiller length (ETL) was measured as were the lamina lengths of a maximum of four live leaves using a ruler. Herbage samples designed to be representative of the grazed horizon were also taken, subsequently oven-dried at 85°C for 18 h and weighed to obtain DM concentration. All sward measurements were taken by the same operator throughout the experiment.

Before and after each test, the micro-sward boxes were weighed to an accuracy of ±0.1 g inside the wind-proof enclosure to minimize air disturbance of the balance (IS1501GG-S0CE, Sartorius AG, Goettingen, Germany). Immediately before the first weighing, the entire exterior surface of each box was wiped clean to remove any soil, water or any other extraneous material in order to ensure that this material did not become detached during the test and bias results.

Each steer was admitted to the arena and remained until the animal had taken ~50 bites from the micro-sward or 5 min had elapsed, whichever came first. The actual number of bites was determined visually/aurally by three observers. Video and audio recordings were also made of each test and the number of bites counted during the test was subsequently verified from the recordings. In the case of a discrepancy in number of bites amongst the observers, the video recording served as the final result. During the test, a companion steer, chosen at random from the other 3 steers, was placed in a pen adjacent to the test arena and was visible to the test steer.

An ungrazed box of the same grass species as that being tested was placed in the same micro-environment as the grazed box and weighed directly before and after each test in order to correct for evapotranspiration losses during the test period. Bite mass was calculated on both fresh and DM bases from the number of bites, the corrected weight change of the boxes and the DM concentration of the herbage.

Statistical analyses

The herbage measurements (ETL, leaf length, SSH, and forage DM), bite number and bite mass data were analyzed as a Latin Square using GenStat (www.vsni.co.uk). There were missing values (a) on day 1 as steer #3 did not eat and (b) a weighing for steer #1 did not store on day 2. Results were also analyzed using the Restricted Maximum Likelihood (REML) directive which fits a variance-components model by residual (or restricted) maximum likelihood (i.e., without using missing values) and were found to be very similar to the general Analysis of Variance (ANOVA) using BLOCK day × steer. All pairwise comparisons were done using Student–Newman–Keuls tests. The distributions for values of the number of bites taken in the tests were examined and found to be normal so transformations were not needed. Simple linear regressions of DM bite mass on ETL, leaf length, SSH, and forage DM were performed.

Results

Over the 4 test days, mean times (h:m, British summer time) for the pre-test weighing of the micro-swards were 09:39 (range 09:28–09:48) for the first

test each day, 11:31 (range 11:26–11:35) for the second, 14:18 (range 14:05–14:44) for the third and 14:59 (range 14:24–15:39) for the fourth test.

Test and control boxes weighed an average of 52.3 ± 1.96 kg and 52.1 ± 1.92 kg before grazing, respectively. Evapo-transpiration losses were 17.6% of the total weight removed during the grazing period.

Herbage data

There were significant effects of grass species on herbage dimensions and DM% (Table 2). SSH was greater for ryegrass than the mean of the other three grass species (17.7 vs. 12.7 cm). ETL was longest for ryegrass and shortest for rough meadow-grass. ETL was greater than SSH for creeping bent, Yorkshire fog and rough meadow grass as would be expected and so the lower value for ETL than SSH for perennial ryegrass is unexpected and probably due to sampling error. Leaf length was longest for ryegrass and Yorkshire fog and shortest for creeping bent. The DM% was lowest for ryegrass and highest for creeping bent and rough meadow-grass.

Number of bites and bite mass

The number of bites taken by each of the 4 individual steers during the tests on each of the 4 test days is shown in Fig. 1. For steers #1 to #4, respectively, the mean number of bites taken on all tests (excluding the zero number of bites taken by steer #3 on day 1) was 34.5, 33.8, 10.7 and 46.3 (F prob. = 0.003). Steer #3 took a low number of bites in all the tests. However,

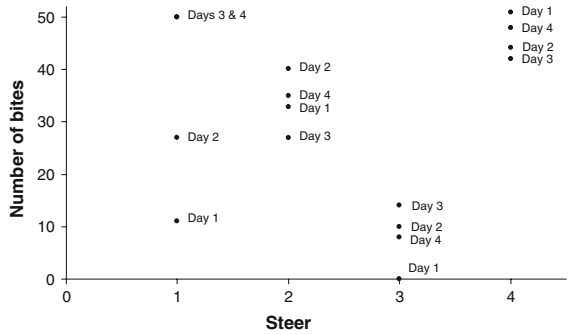

Fig. 1 Number of bites taken by individual steers offered grass species grown in micro-sward boxes in short-term tests

because of the experimental design, in which all steers each were offered all 4 grass species, the mean number of bites taken from each grass species (Table 2) was not significantly different (F prob. = 0.264) and averaged 32.7 bites per box. The mean number of bites taken on each of the test days by the steers was 31.7, 29.8, 33.3 and 35.8 for days 1–4, respectively (F prob. = 0.967). There was no significant relationship between DM bite mass and the number of bites taken in the test (Fig. 2).

Fresh bite mass was greater for ryegrass than the other three treatments (Table 2) and values were 2.62 vs. 1.65 g fresh bite^{-1} (F prob. = 0.024). DM bite mass also tended to be greater for ryegrass (F prob. = 0.057) and values were 0.50 vs. 0.37 g DM bite^{-1}.

Simple linear regressions of mean DM bite mass on the herbage variables are shown in Table 3. These analyses indicated that DM bite mass was not related to leaf length and was only related to ETL, SSH and sward DM% at a low level (R^2 = 0.20–0.25).

Table 2 Sward surface height (SSH), extended tiller length (ETL), leaf length, grass dry matter (DM) %, bite number and bite mass for four grass species grown in micro-sward boxes and offered to individual steers in short-term tests

	Creeping bent	Yorkshire fog	Rough meadow-grass	Perennial ryegrass	s.e.d	F probability
SSH (cm)	13.2^{a}	12.4^{a}	12.6^{a}	17.7^{b}	1.01	0.006
ETL (cm)	13.9^{a}	16.2^{ab}	13.5^{a}	17.3^{bc}	0.94	0.018
Leaf length (cm)	4.0^{a}	7.0^{b}	4.8^{c}	6.5^{b}	0.26	<0.001
DM (%)	22.8^{a}	21.7^{ab}	23.5^{a}	19.7^{b}	0.88	0.030
Number of bites	23.0	30.5	35.3	36.0	7.75	0.400
Bite mass						
(g fresh bite^{-1})	1.65^{a}	1.77^{a}	1.54^{a}	2.62^{b}	0.218	0.024
(g DM bite^{-1})	0.38	0.37	0.37	0.50	0.038	0.057

Means within the same row with different superscripts letters differ ($P < 0.05$)

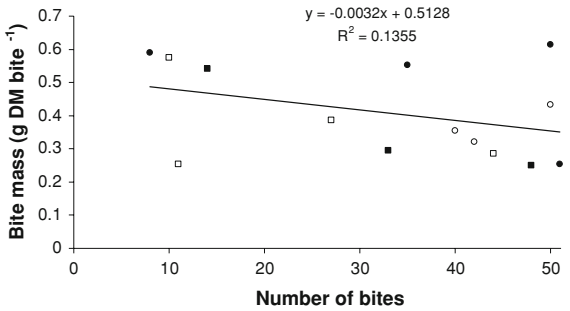

Fig. 2 Bite mass (g DM bite $^{-1}$) and the number of bites taken by individual steers offered creeping bent (□), Yorkshire fog (■), rough meadow-grass (○) and perennial ryegrass (●) in micro-sward boxes in short-term tests

Table 3 Simple linear regressions ($Y = a + bX$) of bite mass (g DM) on independent variables [sward surface height (SSH), extended tiller length (ETL), leaf length, and grass dry matter (DM)] for four grass species grown in micro-sward boxes and offered to individual steers in short-term tests

X	*a*	SE	*b*	SE	R^2	*F* probability
SSH (cm)	0.04	0.202	0.03	0.014	0.23	0.086
ETL (cm)	0.00	0.237	0.03	0.015	0.20	0.110
Leaf length (cm)	0.33	0.170	0.01	0.030	0.02	0.661
DM (%)	1.22	0.401	−0.04	0.018	0.25	0.066

Discussion

Methodology—the role of training and learning

Despite 4 days of pre-trial training (similar to Orr et al. 2005a) and selection of the best four steers (those that most readily took bites from boxes during the pre-trial training) from a group of five steers that had been pre-selected based on temperament and background, not all steers took 50 bites from the boxes within the 5 min period even by the end of the experiment (Fig. 1). Hence the length of training period was probably too short and an outcome of this study is a recommendation that it should be of longer duration before testing begins.

Although the design was powerful enough to demonstrate treatment differences, there was still a large animal to animal variation. For example, Steer #3 had a very low number of bites (10.7 bites vs. a mean of 38.2 bites for the other three steers averaged across all 4 days of the experiment). There was some evidence that steer #1 may have become more used to the test procedure as the experiment progressed. Steer #3 was re-offered the grass species from which no bites were taken on day 1 on day 4 and took 17 bites. Whilst this data has not been included in the analysis (because the Latin Square design would have been compromised), it supports the evidence that some learning was taking place. It could be that four animals was too small a number to safely address this kind of test, particularly if the forage species under study are relatively novel to the grazer. Whilst the four steers had probably already encountered these species while grazing semi-natural pastures, they may not have grazed patches of them before the training period.

Orr et al. (2005a) in a similar study, experienced few problems with animals consuming forage from the micro-boxes after a similar training period. However, one main distinction between that work and the current experiment was that Orr et al. (2005a) worked outside in an environment that was more familiar to the animals. In addition, the animals in that study were able to see behind the boxes (through cattle hurdles) as opposed to the current experiment where the boxes, out of necessity for visual observation and video recording, were placed in a blind corner in a stall inside a barn. The reason for conducting the trial reported here indoors was to eliminate the risk of disruption of the tests by rainfall. This suggests that more training may be needed for this type of work, particularly for animals not accustomed to being handled or to the facilities being used and that consideration should be given to the environment in which the tests are conducted. It is suggested that open-sided structures which provide shelter from rain (e.g., tension fabric canopies) on a hard, dry surface in a pasture setting may provide a suitable testing environment.

Bite mass characteristics of the different species

The results suggest that bite mass in a mixed semi-natural sward would be lower than for a perennial ryegrass monoculture. This might not translate to a lower intake as animals grazing a semi-natural sward may compensate for decreased bite mass by increasing grazing time (Penning et al. 1991a; Rook et al. 1994) or bite rate (Black and Kenney 1984; Barrett et al. 2001). Grazing time has also been shown to increase in

mixed swards compared to monocultures due to an increased search component (Champion et al. 2004). However, animals have a maximum total time available for grazing, as other essential behaviors, such as rumination, resting, grooming, and socialization, must also be completed (Penning et al. 1991b; Rook et al. 1994). Indirect evidence from cattle grazing semi-natural swards suggests that under UK conditions offered at similar levels to those of improved ryegrass-based pastures, DM intakes are also being maintained (Dumont et al. 2007; Isselstein et al. 2007.

Creeping bent in particular tended to have decreased number of bites than the other grass species (mean of 23 bites compared to 34 bites for the other three grass species). This may be due to the stoloniferous morphology of creeping bent (Hubbard 1984), resulting in greater stem material in the bite (as evidenced by lower leaf length in relation to ETL), leading to a need for greater amount of processing (chewing) of each bite and thus leading to fewer bites being taken (Parsons et al. 1994).

Plant factors driving bite mass differences

All species were compared under the same conditions (defoliation at the same time following a 21-day regrowth period). While these results may not be directly transferable to the field, they can be expected to provide useful comparisons between species that are correlated with field results from the same species, based on the evidence of Orr et al. (2005a).

The regression analyses suggests that SSH was related to bite mass ($R^2 = 0.23$), but there was one very high leverage point relating to steer #4 offered perennial ryegrass on the first day of testing. This steer took 51 bites, but the DM bite mass was proportionately 0.43 of that for the other 3 steers when they were offered perennial ryegrass. However, there was no good reason to exclude this value even though if it was removed, R^2 increased to 0.53. These results are in line with previous results where SSH has been manipulated within species (Penning et al. 1994; Prache and Petit 1995). In this case, the SSH variation was mainly due to differences between grass species, as opposed to variation within species, since all grass species were offered to the animals at the same number of days post-cutting. This is supported by an analysis of parallelism which showed that fitting separate lines for each species did not improve the fit.

There was a strong relationship between ETL and SSH, suggesting that in these young, dense, relatively erect swards, ETL and SSH are strongly linked. Bite mass also had a positive relationship with ETL and SSH. Tallowin et al. (1995) examined the same four grass species studied here in grazed pastures and found that perennial ryegrass had longer lamina appearance intervals than the other three species and lamina extension rates were greater for perennial ryegrass than creeping bent or rough meadow grass. Lamina utilization, in terms of percentage of lamina length removed by grazing, was similar between the four grass species.

Bite mass had a negative relationship with forage DM%. For example, perennial ryegrass had the lowest forage DM% but the highest fresh bite mass while rough meadow-grass had higher forage DM% but lower fresh bite mass than perennial ryegrass. However, these results appear to be an artifact of the data because forage DM% also affected DM bite mass. Tallowin et al. (1991) examined in grazed pastures surface water retention, resulting from surface chemistry of the cuticle and the roughness of the lamina surface, for the same four grass species studied here. They found that perennial ryegrass and rough meadow grass had laminae which were highly wettable, unlike creeping bent and Yorkshire fog which they described as having unwettable laminae. These authors also speculated that under wet weather conditions that perennial ryegrass could retain about ten times more surface moisture on the laminae than a pasture dominated by species with less wettable laminae such as Yorkshire fog.

Overall, the low R^2 of the relationships shown in Table 3 resulted from several concurring factors including the variability of slope between species, variability between animals and the non-linearity of the relationships under study.

In mixed swards, species with greater SSH (managed by defoliation—at the same time) could lead to greater apparency of that species. Thus, the grazing animal may consume more material from that species simply because it is more 'visible' due to height, potentially subjecting that species to greater grazing selection than others in the same sward. However, the implications on long-term survival of that species

would be dependent on the residual and sward re-growth characteristics after repeated defoliation.

Additionally, sward bulk density may be perceived as a reward and thereby select patches having higher levels of sward density (Hirata et al. 2006) which may reflect the association of this vegetation characteristic with the rate of DM intake by animals (e.g., Black and Kenney 1984).

Conclusions

This research supports the evidence that sward surface height and extended tiller length influence bite mass of cattle, although the relationships found here were not significant. While we know these characteristic are important for single species, it is imperative that the generality of these characteristics be tested over a range of species, and perhaps most importantly, in mixtures. This is especially important with changed emphasis to unsown swards or swards which are not recently resown and have therefore succeeded towards semi-natural state. The species chosen in this study are particularly relevant to one very widespread example of such a plant community. Gaining a better understanding of the plant-animal interface is key to beginning to comprehend the complexities involved with intake characteristics of grazing animals presented with mixed swards. It was apparent from this experiment that there was a learning curve for the animals to the methodologies used in this experiment. Much is yet to be learned about other factors that may affect bite mass of species found in semi-natural pastures, such as level of maturity, plant density, and plant height within species. In addition, interaction effects arising from growing various mixtures of grasses adds a whole new dimension of complexity related to ingestion due to changes in sward structure. Information gained through studies such as this will eventually lead to a greater understanding of diet selection of grazing cattle, so that pasture management recommendations can be developed to improve the productivity and sustainability of grazing systems.

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