PAPER

An improved grazed class method to estimate species selection and dry matter intake by cows at pasture

Mauro Coppa,[1,2] Anne Farruggia,[2] Philippe Pradel,[3] Giampiero Lombardi,[1] Bruno Martin[2]

[1]Dipartimento di Agronomia, Selvicoltura e Gestione del Territorio, Università di Torino, Italy

[2]Unité der recherches sur les Herbivores, Institut National de la Recherche Agronomique, St-Genès Champanelle, France

[3]Unité expérimentale des Monts-d'Auvergne, Institut National de la Recherche Agronomique, Monts-d'Auvergne, France

Abstract

Research has recently focused on pasture species intake by ruminants due to their influence on animal product quality. A field-applicable method which investigates species intake and selection, was tested on two dairy cow grazing systems: continuous grazing on a highly-biodiverse pasture (C) and rotational grazing on a moderately-diverse sward (R). In addition to the grazed class method, which evaluates the percentage of grazed dry matter (DM) per species according to the residual height of the plant grazed, further measurements were introduced to quantify DM consumption and selection index per species. Six and four representative species were studied in the C and R systems respectively. We found an exponential regression between the presence of a species and its contribution to the cattle's daily intake (P<0.01). On the C plot, *Festuca nigrescens* showed the highest intake (6.2 kg DM/cow d), even if avoided. On the R plot, *Taraxacum officinale* was intensively consumed (6.1 kg DM/cow d), even cows do not express positive selection for the species, while *Poaceae* were avoided. Giving details on species consumption, the improved grazed class method may prove especially useful in non-experimental conditions in biodiverse sward to address grazing management to the consumption of species able to give specific characteristics to dairy products.

Introduction

Grazing is a major inexpensive tool for grasslands conservation management due to its effect on pasture biodiversity and habitat characteristics. Decisions made by grazing herbivores concerning when and where to place bites can lend specific features to structure heterogeneity (Parsons and Dumont, 2003). Selection thus plays a crucial role in grassland dynamics. Research has recently focused on extensive grazing systems, especially in mountain areas, due to their environmental sustainability, their function in maintaining biodiversity and landscape ecology (Cavallero *et al*., 2007), and the peculiarity of the dairy end-products (Martin *et al*., 2005). The interest in mountain cheese and milk quality has focused attention on dairy cows grazing various types of pastures and pasture species (Collomb *et al*., 2002; Tornambe *et al*., 2009). Type and quantity of plant secondary metabolites differ among species (Mariaca *et al*., 1997; Viallon *et al*., 2000) and influence the cows' ruminal metabolism, inducing effects on the nutritional quality of dairy end-products (Leiber *et al*., 2005; Chilliard *et al*., 2007). Hence, the ability to understand the behaviour of dairy cows in terms of pasture species selection offers a strategic management tool for controlling the quality of animal-derived products.

However, the sward complexity of grasslands makes species intake hard to determine. To solve this problem, several methods have been developed based on animal, faeces or vegetation measurements. Methods based on animal observations are effective in giving information on foraging behaviour, but are not usually designed for collecting details on plant species selected and consequently their intakes (Ginane *et al*., 2003; Rutter *et al*., 2004, Rutter 2006). Furthermore, the data is exposed to the influence of observer error (Fraser *et al*., 2006). Several other methods have been developed to quantify the composition of diet consumed by animal grazing heterogeneous grasslands: analysis of *extrusa* collected from esophageally fistulated animals (Fraser and Gordon, 1997), DNA or micro-histological analyses on faeces (Valentini *et al*., 2008; Pegard *et al*., 2009), faecal analysis of long-chain fatty alcohols, long-chain fatty acids and *n*-alkanes (Kelman *et al*., 2003; Ali *et al*., 2004, 2005; Ferreira *et al*. 2009). These methods are however characterized by high analytical costs and complex application, or are not yet developed enough to quantify species intake in non-experimental grazing conditions on highly biodiverse pastures. Vegetation measurement offers an easy way to obtain plant species detail with low analytical costs, also in non experimental contexts, but the methods are not sufficiently developed for estimating species dry matter intake (Orth *et al*. 1998; Hessle *et al*. 2008). Although all these methods can give reliable results, they may prove difficult to apply in more complex environments such as mountain areas where animals can graze on different, highly heterogeneous plant communities.

Among the methods based on vegetation measurement, the grazed class method (Schmutz *et al*., 1963) estimates the percentage of grazed dry matter (DM) per species according to the residual height of a grazed plant. To do this, curves are established to

Corresponding author: Dr. Mauro Coppa, Dipartimento di Agronomia, Selvicoltura e Gestione del Territorio, Università di Torino, via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy.
Tel. +39.011.6708929 - Fax: +39.011.6708798.
E-mail: mauro.coppa@unito.it

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Contributions: MC, AF, PP, BM developed the experimental design; MC, AF, PP also participated to the on-field data collection. All the authors significantly contributed to the interpretation of data and to the revision of drafts prepared by MC, integrating them with their own scientific contribution; AF was particularly involved in the revising work. All the authors approved the final proof. The results about the selection index reported in this work have been already partially presented as a poster at the 15th Meeting of the FAO-CIHEAM Mountain Pasture Network, Les Diablerets, Switzerland.

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describe the cumulative DM distribution in percent along plant height per species. These relationships make it possible to predict the plant DM consumed as a percentage from the residual height of the grazed plant. To realize the curves and facilitate field observations, consumption classes are defined based on percentage of DM per species according to height. Photo-guides are made and used as references to assign grazed plant to the corresponding consumption class. This method has been developed to be easily applied also in non-experimental conditions and used by non-skilled operators. However, it only gives average DM % consumption per plant.

The aim of this study was to improve the grazed class method to obtain a simple, inexpensive and field-applicable estimation of species DM intake and selection and to test the improved grazed class method application to two dairy cow grazing systems on pastures with different biodiversity level in mountain areas.

Materials and methods

Site and grazing systems

The experiment was conducted in 2008 at the INRA's Marcenat farm in an upland area of central France (45°15'N, 2°55'E; altitude 1135-1215 a.s.l.; annual rainfall 1100 mm). The improved grazed class method was applied on two dairy cow grazing systems: continuous grazing (C), set up on a 12.5 ha permanent pasture with a low stocking density (0.96 cow/ha; 1 cow ~ 600 kg liveweight), designed to offer animals a high level of botanical diversity (74 species) and marked structural heterogeneity of vegetation over the season; rotational grazing (R), set up on a 7.7 ha moderately-biodiverse hay-grassland (31 species) plot with a high stocking density (1.56 cow/ha) designed to offer leafy edible biomass throughout the grazing season. This R plot was divided into several paddocks, whose number and size varied during the season to ensure similar herbage availability from one paddock to the next. Stocking density per paddock varied during the season, according to paddock size.

Each system was grazed by 12 multiparous Montbéliarde cows, balanced before the beginning of the experiment for lactation stage, milk production, live weight and body condition scores (BCS) (Table 1). The cows were turned out to pasture from May 21 until September 15 and spent all day stay on the plots except during the morning and evening milkings. No supplementation was provided except for a mineral supplement given *ad libitum* (DM 900 g/kg; Mg 140 g/kg; Na 130 g/kg; Zn 6 g/kg; Mn 5 g/kg; Cu 0.75 g/kg; I 0.06 g/kg; Co 0.02 g/kg).

Vegetation composition (Table 2) was surveyed before the beginning of the experiment (end of May) using the phyto-pastoral approach (Daget and Poissonet, 1969) on six 25-m 50-point transects for each plot. The frequency of each species was registered and botanical composition was expressed by specific contribution (SCi) calculated as the ratio of the frequency of species with the sum of the frequency of all the species in the survey, in percentage. In the C system, dominant grasses were *Festuca nigrescens, Agrostis tenuis, Anthoxanthum odoratum, Avenula pubescens and Dactylis glomerata*; the most abundant legumes were *Trifolium repens* and *Trifolium pratense* and the most abundant forbs were *Achillea* gr. *millefolium* and *Plantago lanceolata.* In the R system, botanical composition revealed the naturalization dynamics of the old original temporary grassland originally composed of *Dactylis glomerata* and *Trifolium repens,* with abundant colonization of *Taraxacum officinale* and invasion by annual or biennial grasses such as *Poa annua, Bromus hordaceus* and *Poa trivialis*.

In addition, species phenological stage was recorded along the transect during each experimental period (Table 3), using the Lambertin' schedule (1990) and the BBCH extended scale (BBA *et al*., 1997).

Table 1. Characteristics of cows involved in the experiment before the beginning of the experiment.

Cow characteristics	Average	SEM
Week after calving	20	1.2
Milk yield, kg/cow/day	17.3	0.91
Cow weight, kg	638	9.2
Body condition score	2.0	0.08

Table 2. Botanical composition of the continuous grazing and rotational grazing plots. Species with specific contribution expressed as the frequency in percentage of a given species i in the vegetation community, higher than 1 are reported.

Continuous grazing			Rotational grazing		
Species	SC, % average	SEM	Species	SC, % average	SEM
Festuca nigrescens	18.4	1.50	*Trifolium repens*	16.9	0.39
Agrostis tenuis	15.3	1.38	*Poa annua*	16.9	2.02
Trifolium repens	7.4	1.01	*Bromus hordeaceus*	16.2	0.95
Achillea gr. *millefolium*	5.3	0.67	*Dactylis glomerata*	14.3	0.13
Anthoxanthum odoratum	4.2	0.87	*Taraxacum officinale*	12.8	0.56
Avenula pubescens	3.3	0.96	*Poa trivialis*	11.3	1.46
Dactylis glomerata	3.1	1.42	*Poa pratensis*	2.7	0.81
Plantago lanceolata	3.0	0.44	*Lolium perenne*	2.7	1.50
Trifolium pratense	2.7	0.50	*Cerastium fontanum*	2.0	0.04
Veronica arvensis	2.4	0.48	*Lamium album*	1.6	0.79
Thymus gr. *serpyllum*	2.4	0.56	*Veronica arvensis*	1.6	0.18
Lathyrus pratensis	2.3	0.66	*Plantago major*	1.0	0.74
Viola tricolor	2.3	0.59	*Rumex crispus*	1.0	0.10
Galium gr. *verum*	2.0	0.36			
Luzula gr. *campestris*	1.9	0.28			
Helianthemum nummularium	1.8	0.99			
Cynosurus cristatus	1.7	0.63			
Festuca gr. *ovina*	1.7	0.56			
Carex montana	1.6	0.58			
Cerastium holosteoides	1.4	0.34			
Rumex acetosella	1.4	0.29			
Daucus carota	1.4	0.41			
Stellaria graminea	1.3	0.53			
Cerastium arvense	1.3	0.29			
Ranunculus gr. *montanus*	1.3	0.32			
Cirsium eriophorum	1.2	0.43			
Lotus gr. *corniculatus*	1.2	0.45			
Potentilla heptaphyllea	1.1	0.50			
Chamaespartium sagittale	1.1	0.56			
Ajuga reptans	1.1	0.39			

SC, Specific contribution; SEM, standard error mean.

Application of the improved grazed class method"

The improved grazed class method was applied at three different periods during the season: early June (P1), early July (P2) and late August (P3). The average of temperature and rainfalls of two weeks (the experimental week and the previous one) were: 9.9°C and 36.8 mm for P1; 12.8°C and 47.2 mm for P2 and 13.9°C and 32.1 mm for P3. These periods matched the evolution of the vegetation in the C plot: vegetative stage in P1, flowering of most abundant dicots in P2, and peak vegetation heterogeneity in P3. In the R plot, the three dates chosen (10 June, 11 July and 27 August) corresponded to the beginning and the middle of exploitation of one of the rotational plots and to two levels of plot stocking density. In P1 surveys were made with a high stocking density (10 cow/ha) in the middle of the plot exploitation (day 5 of 9); in P2 with a low stocking density (5 cow/ha) at the beginning of the plot exploitation (day 3 of 11) and in P3 with a low stoking density (5 cow/ha) in the middle of the plot exploitation (day 6 of 12). This survey scheme enabled us to test the improved grazed class method in different conditions of stocking density (comparing P1 and P3) and level of herbage consumption during grazing events (comparing P2 and P3).

Six species representing the most abundant grasses, legumes and forbs in the C plot were selected for measurements: *Agrostis tenuis, Festuca nigrescens, Trifolium pratense, Trifolium repens, Achillea* gr. *millefolium* and *Plantago lanceolata*. In the R plot, the two most abundant pluriennial grasses and the only abundant legume and forb were selected: *Dactylis glomerata, Poa trivialis, Trifolium repens* and *Taraxacum officinale*. Basing on the recommendations of the statistics guidelines of the *Utilization Studies and Residual Measurements* (Bureau of Land Management's National Applied Resource Sciences Center, 1996), in each measurement period and for each selected species, ten ungrazed plants undamaged by trampling were picked at random along the plots and cut 2 cm from the ground. Outstretched tuft heights were recorded. The plants were then cut into three or four parts and weighed and oven-dried at 60°C to constant weight to determine DM content per sub-sample and total DM per plant (PW_i for a given species i). As *Trifolium repens* is a creeping plant, leaves were collected in a 5 cm^2 area and considered as an individual, without being partly cut. *Achillea* gr. *millefolium* was also partly uncut since this plant was maintained at small-size vegetative stage throughout the season (Table 3). The relation between DM percent distribution in a plant and its height was defined by fitting plant height against cumulative DM% for the ten selected species at the three periods. Figure 1 gives examples of the curves obtained for two species per plot for the three periods. Consumption was assessed using four classes of DM (0, 33, 66 and 100%), with some exception depending on species habit (e.g. for *Trifolium repens* and *Achillea* gr. *millefolium* with prostrate habit only two classes were used: 0 and 100%).

Table 3. Phenology of the studied species during the experimental periods expressed by Lambertin and BBCH codes.

Species	Period 1		Period 2		Period 3	
	Lambertin	BBCH	Lambertin	BBCH	Lambertin	BBCH
Continuous grazing						
Agrostis tenuis	125	13	450	67	725	81
Festuca nigrescens	275	45	625	75	775	93
Trifolium pratense	175	55	450	65	775	93
Trifolium repens	100	29	400	64	725	89
Achillea gr. *millefolium*	125	51	150	55	100	29
Plantago lanceolata	375	65	575	75	775	93
Rotational grazing						
Dactylis glomerata	275	45	150	19	125	13
Poa trivialis	300	49	175	40	225	43
Trifolium repens	100	29	400	64	250	44
Taraxacum officinale	575	75	100	29	100	29

Period 1, early June; Period 2, early July; Period 3, late August. Lambertin codes (G, grasses; F, forbs): 100 = G/F, vegetative stage; 200 = G, 70% of spikes in stems; F, 70% of flowering bottoms present; 300 = G, 70% of spikes out of stems, spikes close to stems; F, 70% of flowering bottoms opened; 400 = G/F, flowering stage; 500 = G, lactic corns; F, flowers withered; 600 = G, doughy corns; F, starting fructification, fruits formed; 700 = G, hard corns; F, fruits fully ripened; 800 = G/F, end of vegetation.BBCH code: 0-10, germination, sprouting out, bud development; 10-20, leaf development; 20-30, formation of side shoots/tillering; 30-40, stem elongation/shoot development; 40-50, vegetative propagation/booting; 50-60, inflorescence emergence/heading; 60-70, flowering; 70-80, development of fruits; 80-90, ripening or maturity of fruits and seeds; 90-100, senescence, beginning of dormant.

Photo-guides were made for each species during each period: an entire plant representative of the average height of a given species was selected in the plot and cut at the height corresponding to the consumption classes established. A picture of each class was taken. The DM percent consumption (DMC%) was then determined for each selected species at each measurement date along the same transects used for the vegetation surveys. Consumed DM in kg (Sp_i) per plant was calculated for a given species i, as follows:

$$Sp_i = PW_i \times (DMC\%_i/100)$$

where PW_i is the total weight of plant DM (in kg) for given species i.

Additional measurements were made to improve the grazed class method. When collecting the ten plants per species, we measured the basal area (A_i) of each collected plant, and assigned a value that was a multiple of a 0.5 cm side square (starting a form 2×2 cm square). These measurements enabled us to calculate average daily individual species DM intake for each chosen species i (DMI_i). DMIi (kg of DM/cow day) was estimated by multiplying the consumed DM per plant (Sp_i) by the estimated number of plants of species i in the plot (n_i) divided by number of grazing days from the beginning of the given grazing event (d) and number of dairy cows (c), using the following equation:

$$DMI_i = (Sp_i \times n_i)/(c \times d)$$

The estimated number of plants of species i on the plot (n_i) was calculated dividing the paddock area (in ha) (A) by the average area covered by a single plant of species i (in ha) (A_i), obtaining then an estimation of the number of plant present on the plot of an hypothetical monospecific pasture of the i specie. Multiplying it by the specific contribution of species i (SC_i), using the following equation:

$$n_i = (A/A_i) \times (SC_i/100)$$

it was possible to estimate the estimated number of plants of species i (n_i) on the plot.

For the continuous grazing system, the DMIi between two successive periods was calculated as follows:

$$DMI_i = [(Sp_i \times n_i)_{p2} - (Sp_i \times n_i)_{p1}] \times [(d_2 - d_1)/c]$$

where $_1$ and $_2$ indicate two successive survey periods.

At the same time, average potential intake capacity (PIC, expressed in kg of DM/cow day) of the cows was calculated for each period, taking into account milk production, live weight, age, body condition score, lactation stage and gestation week of each cow according to the equations given in Faverdin *et al*. (2007). The contribution of each species to PIC (%PIC) was then evaluated as the ratio of DMI_i to PIC.

In addition, a selection index (SI) for each period was calculated per species according to the *taux d'abroutissement* method (Orth *et al*., 1998), using the following equation:

$$\text{Selection index } (SI_i)=[(DMC\%_i \times SC_i)/\Sigma(DMC\%_i \times SC_i)]/(SC_i/\Sigma SC_i)$$

Species *i* was selected if SI*i* was greater than 1, not selected if SI*i* =1 and avoided if lower.

Statistics

Statistical analyses were performed using SPSS for Windows software (version 13.0; SPSS Inc., Chicago, IL, USA). For all the analyses, transects were taken as the statistical unit. To evaluate seasonal evolution of DMI_i and SI*i* per species, we ran repeated-measures ANOVA with period as within-subject factor for the continuous grazing system, where surveys were repeated along the same transects during different periods of the same grazing event. A GLM ANOVA with period as fixed factor was performed to test the effects of paddock stocking density and level of herbage consumption during grazing event for the rotational grazing system, where each grazing event was concluded when cows were moved to another paddock. The subsequent grazing event started from approximately the same herbage situation as the previous one, so that grazing events could be considered independent each other. On averaged across-season data of species DMI_i, species effect was tested by a GLM ANOVA for grazing system. All *post-hoc* comparisons were performed using the REGWQ test. For each species, SI deviation from 1 was evaluated using a Student's *t*-test. The effect of SC (independent variable) on PIC% (dependent variable) was tested by an exponential regression using the Enter method.

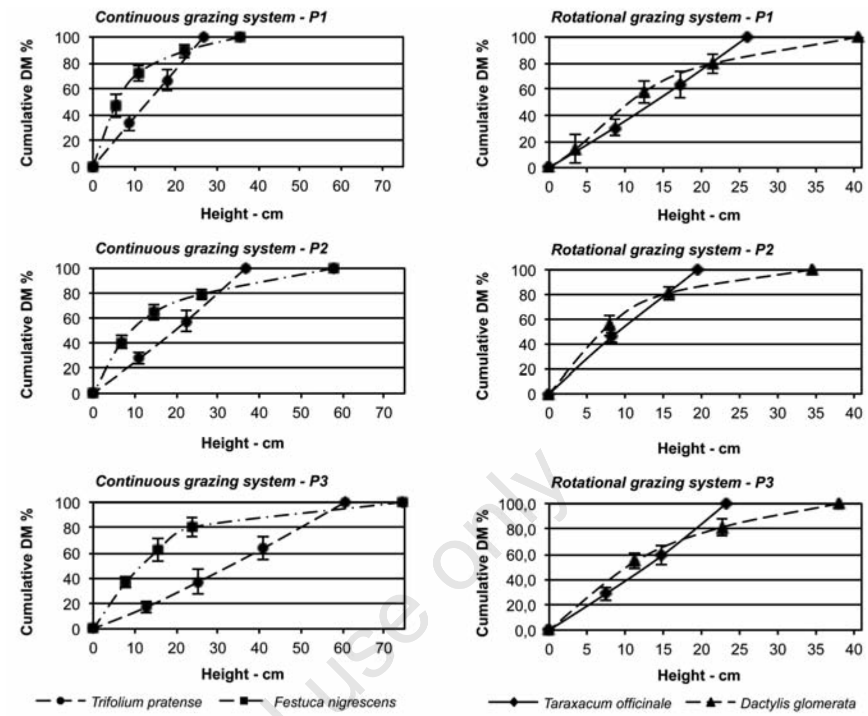

Figure 1. Plant dry matter (DM) distribution (%) in relation to height (cm) during the experimental periods (P1, early June; P2, early July, P3: late August): examples of curves for *Festuca nigrescens* and *Trifolium pratense* for the continuous grazing system and *Dactylis glomerata* and *Taraxacum officinale* for the rotational grazing system.

Table 4. Cow performance for each experimental period and for season.

Cow performance	Period 1	Period 2	Period 3	SEM	Period effect
Rotational grazing					
Cow weight, kg	620	624	651	5.4	ns
Body condition score	1.69	1.85	2.03	0.034	ns
Milk yield, L/cow×day	18.0	18.5	15.5	0.27	ns
Continuous grazing					
Cow weight, kg	619	644	645	4.6	ns
Body condition score	1.72	1.59	1.64	0.036	ns
Milk yield, L/cow×day	21.3^a	18.5^b	11.4^c	0.31	***

Period 1, early June; Period 2, early July; Period 3, late August; ns, not significant; ***P<0.001; a,b,c values with different superscripts within the same row are significantly different.

Results

No differences were found among period for cow body weight and BCS in both R and C grazing systems. Milk yield remained quite stable for R cows, while decreased progressively during the season for C ones (Table 4).

A significant exponential regression was found between specific contribution (SC) (x) and contribution potential intake capacity (PIC%) (y) of the all selected species of the two plots ($y=2.8853e^{0.1192x}$; $R^2=0.706$; P=0.002). There were no seasonal changes in species DM intake (DMI_i) for all the studied species in the continuous grazing system. The effects of paddock stocking density and level of herbage consumption according to the paddock utilization rate on DMI_i in the rotational grazing systems were not significant. On the continuous grazing system, and throughout the grazing season, *Festuca nigrescens* was the most heavily consumed species (6.24 kg of DM/cow day; P<0.001; Table 5). DMI_i was 3 and 6-fold higher for *Festuca nigrescens* than *Agrostis tenuis* (1.98 kg of DM/cow day) and *Trifolium repens* (0.64 kg of DM/cow day), respectively. No differences were observed between *Trifolium pratense* (1.27 kg of DM/cow day), *Achillea* gr. *millefolium* (1.01 kg of DM/cow day) and *Plantago lanceolata* (0.76 kg of DM/cow day). Pooled together, the six selected species contributed 59.9% of the cow's PIC, estimated at 20.0±0.17 kg of DM/cow day for C cows. The PIC% of selected grass, legumes and forbs were respectively 41.0%, 9.6% and 8.9%. In contrast with DMI results, *Festuca nigrescens* was avoided, while *Trifolium repens* and *Achillea* gr. *millefolium* were selected (SI: 0.71, 1.24 and 1.35, respectively; Table 5). None of the other species were selected or avoided. There was a significant period effect on the SI of *Festuca nigrescens*, which was selected against throughout the season, but especially during P1 (SI=0.47 in P1 vs. 0.79 in P2 and 0.85 in P3; P=0.013). An opposite trend was observed for *Trifolium repens* (P=0.054), which was not selected in P1 (SI=0.83) but was selected in P2 and P3 (SI=1.33 and 1.55, respectively).

On the rotational grazing system, *Taraxacum officinale* was the most heavily consumed species in terms of DMI_i (6.05 kg of DM/cow day, P<0.001; Table 5) while *Poa trivialis* was the less consumed (2.06 kg of DM/cow day). The DMIi of the two selected grasses was 1.8-fold lower than the DMIi of the two studied dicotyledons (5.86 and 10.53 kg of DM/cow day, respectively). The PIC% of the grasses and dicotyledons studied were 28.0 and 50.4, respectively, and the four species contributed to 78.4% of ingestion capacity, estimated at 20.9±0.16 kg of DM/cow day for R cows. The studied grasses were selected against, while *Taraxacum officinale* was not selected and *Trifolium repens* was positively selected (Table 6). *Trifolium repens* selection increased during the grazing season (SI=1.25, 1.55 and 1.58 in P1, P2 and P3, respectively; P=0.007). A period effect was observed for *Dactylis glomerata* (P=0.011), which was not selected in P1 and was selected against in P2 and P3 (SI=1.01, 0.68 and 0.67 in P1, P2 and P3, respectively).

Table 5. Estimation of the average species dry matter intake, contribution to the potential intake capacity, and specific contribution.

Species	Intake, kg DM/cow/day average	Contribution to PIC, %	SC, % average
Continuous grazing			
Agrostis tenuis	1.98^{B}	9.9	15.3
Festuca nigrescens	6.24^{C}	31.2	18.4
Trifolium pratense	1.27^{AB}	6.4	2.7
Trifolium repens	0.64^{A}	3.2	7.4
Achillea gr. *millefolium*	1.01^{AB}	5.1	5.3
Plantago lanceolata	0.76^{AB}	3.8	3.0
Total	11.97	59.9	36.2
Standard error	0.351		
Species effect	***		
Rotational grazing			
Dactylis glomerata	3.80^{B}	18.2	14.3
Poa trivialis	2.06^{A}	9.9	11.3
Trifolium repens	4.48^{B}	21.4	16.9
Taraxacum officinale	6.05^{C}	28.9	12.8
Total	16.38	78.4	55.3
Standard error	0.333		
Species effect	***		

PIC, potential intake capacity, calculated according to Faverdin *et al.* (2007); SC, specific contribution; DM, dry matter; ***P<0.001; ^{A,B,C}values with different superscripts within the same row are significantly different.

Table 6. Species dry matter selection indices for the studied species for each experimental period and for season.

	DM selection index					
Species	Period 1	Period 2	Period 3	Season	SEM	Period effect
Continuous grazing						
Agrostis tenuis	0.97	0.95	0.92	0.95	0.020	ns
Festuca nigrescens	0.47$^{A\#}$	0.79$^{B\#}$	0.85$^{B\#}$	0.71	0.013	*
Trifolium pratense	0.91	0.87	0.93	0.90	0.029	ns
Trifolium repens	0.83	1.33B°	1.55$^{B\circ}$	1.24$^{B\circ}$	0.029	*
Achillea gr. *millefolium*	1.29°	1.36°	1.41°	1.35°	0.047	ns
Plantago lanceolata	0.99	1.06	1.02	1.02	0.049	ns
Rotational grazing						
Dactylis glomerata	1.01^{B}	0.68$^{\#}$	0.67$^{\#}$	0.77$^{\#}$	0.057	*
Poa trivialis	0.60$^{\#}$	0.55$^{\#}$	0.66$^{\#}$	0.60$^{\#}$	0.032	ns
Trifolium repens	1.25$^{A\circ}$	1.55$^{B\circ}$	1.58$^{B\circ}$	1.48°	0.053	**
Taraxacum officinale	0.97	0.96	0.99	0.97	0.051	ns

P1, early June; P2, early July; P3, late August. DM, Dry matter; ns, not significant. *P<0.05; **P< 0.01; ^{A,B,C}values with different superscripts within the same row are significantly different; °indicates items selected; #indicates avoided items.

Discussion

The improved grazed class method yielded data on the intake and selection of some chosen species in two contrasted mountain grazing systems. The method gave results in a fairly simple sward situation, as in the rotational grazing system, as well as in highly complex vegetation communities, as in the continuous grazing system. Data were obtained either for studied species with high or low specific contribution (SC). This makes this method suitable for application to not abundant species, but whose richness in secondary metabolites could affect the cows' ruminal metabolism, inducing effects on the milk fatty acid (FA) profile (Leiber *et al*., 2005; Chilliard *et al*., 2007). Relations between milk FA profile and botanical composition of mountain pastures have been thus showed by Collomb *et al*., (2002). Differences in cheese flavour, aroma, and especially in texture have also been observed according to pasture type exploited by cows (Martin *et al*., 2005; Coppa *et al*., 2011). Selection index was able to give useful information on the cows' foraging behaviour in both grazing systems, confirming behaviour patterns already observed in similar situations. In particular, in continuous grazing systems, where vegetation is characterized by highly heterogeneous species distribution and nutritive value, sward conditions allow cows to select the more palatable species at an early phenological stage and avoid lower-quality patches, especially at the beginning of the season (Dumont *et al*., 2007b). During the season, cows usually return to previously grazed areas, where they patch-graze (Alder *et al*., 2001), and choose just to walk across the less fertile areas (Coppa *et al*., 2009). This foraging behaviour could explain why, in our experiment, the cows selected against *Festuca nigrescens*, especially at the beginning of the grazing season, when vegetative patches of other more palatable grasses such as *Dactylis glomerata* (Cavallero *et al*., 2007) were available in the sward. This selection pattern could also explain why the contribution to potential intake capacity (PIC%) of the studied species was just a little less than 60% even if we choose six of the dominant species. However, the relation between SC and PIC%, already shown by Carpino *et al*. (2003), makes the DM intake of *Festuca nigrescens* the highest among the species analyzed in this study. Previous reports have asserted that the cost-benefit ratio would not be favourable if animals tried hard to avoid dominant grassland species (Thornley *et al*., 1994; Parson and Dumont, 2003). On this basis, *Trifolium repens*, a small-sized plant often mixed with other species in the patches, was not selected at the beginning of the grazing season. Patch grazing was, however, favourable to prostrate and small-sized plants (Grime *et al*., 1988) with high regrowth rate, resulting from selection of Trifolium repens growing throughout the season. The overgrazing of *Achillea* gr. *millefolium* in previously-grazed patches and its rapid regrowth rate could also explain its selection and its vegetative phenological stage throughout the season. The seasonal dynamics in cows' selection throughout the season could be a way for cows to regulate their species intake, resulting in no difference on species DM intake among periods.

In the R system, the application of the improved grazed class method showed high dicots consumption compared with grasses consumption, together with changes in selection indices according to paddock stocking density and level of herbage consumption in relation to paddock utilization rate. This could be explained by a combined effect of different relations between height and DM distribution among species and by the cows' consumption patterns. Indeed, in rotational grazing, cows have minimal scope for selection and are forced to consume herbage, grazing thus vegetation *by stratum* (Morris, 2002; Teague and Dowhower, 2003). *Poaceae* species, whose DM was more concentrated at the base of the plant (Figure 1), turned out to be selected against, especially at early grazing stages. This effect was reduced over time and with increasing stocking density. For the same reason, *Taraxacum officinale*, showing a more regular DM-height distribution, was heavily consumed. *Trifolium repens* selection could be explained by two factors: rapid regrowth, which seems favorable in intense grazing conditions (Grime *et al*., 1988; Dumont *et al*., 2007a), and cows' preference for this species over *Poaceae*, as demonstrated by Rutter *et al*. (2004; Rutter, 2006) on conterminal monocultures. Herbage exploitation by stratum could also contribute to explain why in our trial the four studied species counted for about the 80% of the total PIC.

The application of the improved grazed class method also carries limitations. The limits of the initial grazed class method (Schmutz *et al*., 1963) were presented by the Bureau of Land Management's National Applied Resource Sciences Center (1996). DM-height relations and photo-guide preparations are cumbersome and time-consuming. Several guides may need to be developed for each key species in order to match broad year-to-year or site-to-site variations in growth forms (Gierish, 1967). This means large datasets have to be put together, as it have been done by the Bureau of Land Management's National Applied Resource Sciences Center in USA, in order to use this method for ordinary surveys and for land management. Most of the American pasture species being different form European ones and growing in different environmental condition, a European dataset should be built up. It is in any case necessary to individuate useful agronomical and climatic variables (e.g. irrigation, level of fertilization, precipitation and cumulative temperature) suitable for Europe, to select the guide closest to plot characteristics. Even though the class approach, for both the photo-guide and the plant area measurement proposed in the present work, makes this method easily applicable by non-scientific operators, it can introduce a degree of approximation in estimating species DM intake. This approximation could have confused period effect. On the other hand, the lack of differences on species DM intake during the season could be supported by the stability of cow body weight and BCS. Changes in milk yield could be better explained by changes in herbage quality rather than quantity. Cows could also regulate their species DM intake throughout the season or according to stocking density or level of paddock exploitation conditions by selection, as showed by the SI result. Being SI derived directly by the grazed class method data without further approximation, it could be more sensitive to foraging behaviour changing. There was a generally appreciable variability in the data used to establish the relations between DM distribution and plant height obtained from the ten sampled plants per species. The method would be optimized by increasing the number of plants sampled to improve the reliability of the curves. However, the number of plants chosen in this work (ten) offered a good compromise between reliability of the results, facility of the work, and field-applicability of the method. That said, the choice of the ten plants in the sward could have a relevant effect on the DMI results. It is important to select plants representative of the average situation of the studied specie in the plot (height, size and phenology). Operatives should walk across the plot before starting the plant sampling process to be able to sample plants representatives of the average situation of the whole plot. It is recommended the operatives train themselves by running samplings in different plots and situations before starting measurement on the plot targeted for study. The PIC calculated by the equation proposed in Faverdin *et al*. (2007) is inexpensive and easy to calculate, but the data generated is not as precise as with analytical methods, such as faecal analysis (Kelman *et al*., 2003; Ali *et al*., 2004 and 2005; Fraser *et al*., 2006, 2009; Ferreira *et al*., 2009). Selection indices, which are calculated based on DMI%, are also affected by these limits, which further suggests that this method may not always give highly precise data. Even so, our results demonstrated the sensitivity of the proposed method in revealing foraging behaviour patterns already measured in the literature by other animal observations and sward measurement-based methods without species details (Morris 2002; Teague and Dowhower 2003; Hülber *et al*., 2005; Farruggia *et al*., 2006; Dumont *et al*., 2007b). Furthermore, the methods based on the direct observation of animal behaviour on biodiverse pastures give only distinction-based selection indices for grasses, legumes and forbs (Farruggia *et al*., 2006; Dumont *et al*., 2007b) as the observer cannot tell by observation only which species an animal is eating when it is grazing an intimately-mixed sward (Rutter 2006). With only slightly more work, our method is able to produce estimates of DM intake per species. Only a handful of studies on vegetation-based methods have arrived at the same result (Carpino *et al*., 2003), and with more time-consuming methods.

Non vegetation-based methods could give more precise data, but are either more invasive (fistulation) for the animals (Fraser and Gordon, 1997) or need laboratories specialized in expensive, cumbersome analysis. There are numerous faeces analysis-based techniques for producing information on animal diet. Micro-histological analysis identifying cellular structure of plant wall material patterns by comparison with known plant reference material gives good results in the characterization of animal diet at family or species level (Hessle *et al*., 2008). However, this method does yet allow quantification, because it does not take into account variation in rate of digestion of different plant species or plant parts (Fraser *et al*., 2006). Recently, some methods have been developed based on plant DNA identification in faeces to characterize diet composition at species level, with encouraging results (Valentini *et al*., 2008; Pegard *et al*., 2009), but are not enough advanced to enable per-species intake quantification. The analysis of *n*-alkanes, long-chain fatty alcohols and long-chain fatty acids in faeces as diet composition markers has recently been improved to quantify intake at species level (Ali *et al*. 2005) and applied to animals either consuming controlled simple diets (Kelman *et al*., 2003; Ali *et al*., 2004, 2005; Ferreira *et al*. 2009) or grazing moderately-diversified swards (Fraser *et al*., 2006, 2009), with encouraging results. Even so, these methods require preliminary investigation of the *n*-alkane, long-chain fatty alcohol and long-chain fatty acid composition of all the species present in the sward grazed, together with rate of digestion of the different species. This makes these methods cumbersome and difficult to use in very complex, heterogeneous and highly biodiverse sward conditions, such as extensive mountain pastures.

Conclusions

The improved grazed class method proposed in this study is an inexpensive and easy-to-apply method for characterizing the diet composition of free-ranging animals. It is equally accessible to non-scientific operatives, and can be applied in non-experimental conditions on either moderately-biodiverse swards or highly complex, highly biodiverse mountain pastures, as shown by the results of our applications. Moreover, the proposed improvements can give estimates of DM intake per species with only slightly more work. However, large datasets have to be built up before this method could be used for ordinary surveys and for land management and further application would be useful to test our method in other different contexts.

Daily per-species DM intake by cows provides valuable data for studying biodiversity management and relations between vegetation and the derived dairy end products. The inexpensive and easy application of the improved grazed class method, making it accessible to non-scientific operatives, could favour its application in farm-scale professional studies aiming to ameliorate dairy cows pasture feeding. Collecting data about species intake on complex swards could be an important instrument to address grazing management to consumption of those species able to give specific characteristics (nutritive and sensory) to derived dairy products, valorising their linkage with the production *terroir*.

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