ELSEVIER

Animal Feed Science and Technology
84 (2000) 49–68

ANIMAL FEED SCIENCE AND TECHNOLOGY

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Vertical distribution of biomass, chemical composition and pepsin—cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day

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Received 28 December 1998; received in revised form 13 December 1999; accepted 10 January 2000

Abstract

The vertical distribution of biomass, chemical composition (crude protein (CP); neutral detergent fibre (NDF); acid detergent fibre (ADF); acid detergent lignin (ADL); total soluble carbohydrates (TSC)) and organic matter digestibility estimated from pepsin-cellulase (PCOMD) of a perennial ryegrass sward was studied under strip-grazing management. Measurements were carried out during three grazing cycles (May, June and October) for three regrowth ages in each month (21, 28 and 35 days) and at two times in the day for each of these ages (08.00 and 19.00 h). Each grass sample was cut in four layers (0–5, 5–10, 10–15 and >15 cm), then freeze-dried, ground and analysed.

Very large vertical gradients were observed in the different chemical constituents, with the following mean variations from the upper to the lower layer of the sward: +80 g DM/kg fresh grass, −100 g CP/kg OM, −30 g TSC/kg OM, +250 g NDF/kg OM, +22 g ADL/kg OM and −25 units PCOMD (%). These variations in chemical composition linked to height in the sward were often of greater magnitude than the variations measured on the whole plant for each month, regrowth age or time of day. For a given chemical constituent, the ranking between layers was unaffected by season, regrowth age or time of day, except in the case of TSC. However, the differences in contents of certain constituents between layers were sometimes seen to vary strongly with the month (bulk density, CP, TSC), regrowth age (CP) or time of day (TSC). The vertical distribution of NDF, ADL and PCOMD showed relatively little variation with month, regrowth age or time of day within the vegetative stage studied.

Equations were established which relate chemical composition to height in the sward. From these results, it is possible to simulate the influence of sward defoliation depth on the chemical

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PII: S0377-8401(00)00114-0

composition of the diet selected by grazing ruminants.

Keywords: Perennial ryegrass; Vertical structure; Chemical composition; Pepsin–cellulase digestibility; Season; Regrowth age; Time of day

1. Introduction

The amount of any chemical constituent of herbage ingested by a grazing ruminant is the product of the herbage intake by the constituent content of the grass selected by the animal. The chemical composition of the selected grass depends not only on the pre-grazing chemical composition of the grass, but also on the herbage allowance which determines the degree of vertical and/or horizontal diet selection.

Numerous studies have shown that the chemical composition of grass is influenced by the season of growth and age of regrowth (Wilman et al., 1976; Demarquilly and Andrieu, 1988), as well as by the time of day (Holt and Hilst, 1969; Lechtenberg et al., 1972). Under strip-grazing management, defoliation of the sward takes place by successive layers from the top of the canopy (Wade, 1991), so the chemical composition of the selected grass is a function of the vertical distribution of the chemical constituents in the sward. In order to predict the chemical composition of the ingested herbage, it is necessary to know this vertical distribution and the main factors controlling its variation.

In grass species, it is well established that the bulk density of the sward increases with the depth of the sward, as well as the proportion of sheaths, stems and dead tissue (Wilkinson et al., 1970; Clark et al., 1974). The leaf blades are generally more digestible, richer in crude protein and poorer in cell-wall constituents than sheaths and stems (Deinum and Dirven, 1975; Wilman et al., 1976). There is, thus, an increasing or a decreasing vertical gradient of composition according to the chemical constituent. Such a vertical gradient in composition has been described in tropical grasses (Wilkinson et al., 1970; Stobbs, 1975; Hendricksen and Minson, 1980; Herrera et al., 1984) and on grass/legume mixtures (Clark et al., 1974; Holmes et al., 1992; Johnston et al., 1993; Wilkins et al., 1995). Very few data are available on pure temperate grasses under rotational grazing. In particular, there are few descriptions in the literature concerning the vertical gradient of chemical constituents in swards of perennial ryegrass (*Lolium perenne* L.), which is certainly the most widely used grass species for grazing in temperate environments. Similarly, the factors controlling the vertical variation of chemical composition in swards have often been the subject of separate studies (Wilkinson et al., 1970; Herrera et al., 1984; Johnston et al., 1993).

The aim of the present study is, thus, to describe the changes in vertical distribution of the biomass, chemical composition and digestibility of a perennial ryegrass sward during several months of the year, at different regrowth ages and at different times of the day. The practical aim is to predict the chemical composition of the herbage ingested by ruminants under different grazing conditions (stocking rate, season, regrowth age).

Table 1
Date of grazing, date of herbage sampling, and regrowth age of the sward for the nine days of measurement

Date of grazing	Date of sampling	Cycle number	Regrowth age		
			Days	Cumulative degree-days[a]	Radiation (MJ/m^2)
12–15 April	08 May	2	23	275	296
	15 May	2	30	344	411
	22 May	2	37	423	531
16–17 May	07 June	3	21	280	371
	14 June	3	28	371	492
	21 June	3	35	487	615
18–19 June	–	–	–	–	–
21–22 July	11 October	5	24[b]	344	192
	18 October	5	31	449	247
	25 October	5	38	535	296

[a] Cumulative degree-days are calculated from the first day of regrowth (basis: 0°C).
[b] Autumn regrowth was stated to start on 17 September after rain (no residual herbage at that time).

2. Materials and methods

2.1. Experimental site

All the herbage sampling was carried out in the spring and autumn of 1995 on a 1-ha paddock at the Méjusseaume experimental farm (INRA Rennes, France). The soil is loamy with a pH of 6.0, being also poor in organic matter (2%) and sensitive to water stress, particularly during the summer.

The paddock was sown in September 1992 with perennial ryegrass cv. Belfort. From 1993 to 1995, the paddock was strip-grazed by the dairy cow herd with ≈28 day-intervals between grazing passages in spring and greater intervals in summer and autumn. In spring 1995, 60 kg N/ha was spread after each period of grazing, as given in Table 1. After the fourth herd passage in late July, grass growth rate stopped during August and started again in September with the first periods of heavy rain (78 mm from 7 to 17 September).

2.2. Experimental design and measurements

Herbage samples were taken only on regrowths, in Cycle 2 (May), Cycle 3 (June) and Cycle 5 (October). Cycle 1 (i.e. primary growth) and Cycle 4 in summer were not investigated because of their expected very low grass growth rate and difficulties in defining clearly the regrowth ages. For each studied month, a non-replicated area of 5 m×6 m was fenced off about two weeks before the first measurements in order to investigate regrowth age independently of the subsequent dates of passage of the herd. At each month, the fenced area was moved and a new fenced area was chosen close to the previous fenced area, on the basis of being well grazed, as homogeneous as possible and containing a few cow dungs. The three fenced areas were situated at distances of only 20–30 m from each other. It was, thus, hypothesized that differences between months, if they occurred, would be related less to spatial heterogeneity within the paddock than to actual differences between phenological state of the grass, season of growth and number of grazing passages.

For each of the three studied months, grass sampling was carried out at 3, 4 and 5 weeks of regrowth (Table 1). For each of the nine measuring dates (3 months$\times$3 regrowth ages), two grass samples were taken in the morning starting at 07.45 h and two others in the evening at 18.30 h. Regrowth age and time of day were randomly investigated within each month. At each sampling, a quadrat of 60 cm$\times$60 cm was placed on the ground at random within the whole fenced area, just avoiding the cow dungs. Tillers taking root outside the quadrat were rejected. Within the quadrat, the extended height from ground level of the highest leaf blade and sheath was measured on 10 tillers chosen at random using a graduated rule. Grass was then cut with scissors down to ground level over the entire quadrat. Handfuls were placed in a container, taking great care to maintain the vertical structure of the sward. A first sample was kept cool in an ice-box during the collection of the second sample. The time required to take both samples averaged about 50 min.

As soon as possible, each fresh sample was cut into four layers (0–5, 5–10, 10–15, and +15 cm, denoted here, respectively, as layers 1, 2, 3 and 4). Each layer was weighed as fresh and immediately deep-frozen to -20°C. The average time between the end of sampling in the field and deep freezing was 40 min. Samples were then freeze-dried and weighed on removal from the freeze-dryer in order to estimate the dry matter content (DMf) of the grass. Preliminary tests on fresh grass showed that DMf is closely related to the DM content as measured by oven-desiccation for 48 h at 80°C (DMf (%)=0.76+0.98 DM oven (%); n=36; R^2=0.98, rsd=0.63). Each sample was then ground to pass a 0.8-mm screen before chemical analysis.

2.3. Chemical analysis

All grass samples were analyzed for ash, total nitrogen (N) and cell-wall constituents (NDF, ADF, ADL). The two replicates were pooled together at equal weights for the determination of total soluble carbohydrates (TSC) and pepsin–cellulase digestibility of dry matter (PCD). The ash content was determined by ashing at 550°C in an oven for 5 h (AFNOR, 1985). Nitrogen was analyzed using the Kjeldahl method (AFNOR, 1985), the distillation and titration being performed automatically by a Buchi 322 machine (Roucaire, France). The NDF, ADF and ADL contents were determined according to the method of van Soest (1963) adapted for a Fibertec analyzer (Tecator) as described by Giger and Pochet (1987). The TSC were analyzed using the Luff–Schoorl method after hydrolysis for 30 min in 0.1 N HCl (AFNOR, 1985). The pepsin–cellulase digestibility was estimated according to Aufrère and Michalet-Doreau (1988), with a three-stage technique: pre-treatment with pepsin in hydrochloric acid (0.2% pepsin in 0.1 N HCl), starch hydrolysis, attack by cellulase (Onozuka R 10 from *Trichoderma viride*, Yakult Honsha, Japan). An estimate of in vivo OM digestibility (denoted PCOMD) was then calculated from pepsin–cellulase digestibility using the following equation proposed by Aufrère and Demarquilly (1989) for fresh grass pastures, which relates the actual in vivo OMD to PCD with a good degree of accuracy:

$$\text{in vivo OMD}\,(\%) = 0.720\ \text{PCD}\,(\%) + 23.36;\quad \text{rsd} = 1.99;\ R^2 = 0.94;\ n = 85$$

2.4. Calculations and statistical analysis

The contents in CP, NDF, ADF, ADL and TSC were always expressed as g/kg OM in order to remove possible contamination from soil, especially in Layer 1. The bulk density in each layer, expressed in kg OM/ha/cm, was calculated as the ratio between the biomass and the mean height of the layer considered. This height was taken as 5 cm for layers 1, 2 and 3, while the difference between the mean extended height and 15 cm was applied to Layer 4.

The experimental scheme was a randomized block design, with each month considered as a block, with regrowth age and time of day randomized within each month. As the effect of month is presumably dependent on the effects of regrowth age and time of day, 'month' was considered as a statistical treatment in order to test interactions of month with age and time as in a completely randomized design (Gill, 1986). However, since months were mixed indiscriminately with fenced areas, the main effects of month per se could not be investigated.

All parameters were thus treated by variance analysis according to a model taking account of the effects of month, regrowth age and time of day, as well as month×age, month×time of day and age×time of day, using the GLM procedure of SAS (1987). The variance analysis was carried out on repeated time (layers 1–4) with the polynomial option of the GLM procedure of SAS (1987). The linear, quadratic or cubic power variation of each constituent with the height in the sward could thus be tested, as well as the interactions of the effect of layer with month, age, time of day, month×age, month×time of day and age×time of day.

3. Results

3.1. Height, biomass and bulk density

The extended tiller height was significantly higher in October than in May and June (+11.8 cm), and sheath height was slightly higher in June than in May and October (+1.2 cm) (Table 2). The extended tiller height increased with regrowth age by 0.7 cm per day on average, whatever the month. The rate of elongation of sheaths was higher in June (0.7 cm per day) than in May (0.3 cm per day) and October (0.2 cm per day), indicating the presence of growing stems with internode elongation. At 21 days of regrowth, Layer 4 only contained leaf blades, whatever the month. At 28 days of regrowth, the percentage of tillers with sheaths exceeding a height of 15 cm was 0, 10 and 0 in May, June and October, respectively. At 35 days of regrowth, the corresponding result was 15, 70 and 5%.

Table 2
Effect of month, regrowth age and time of day on sward height and on the vertical distribution of biomass, bulk density, DM and OM contents in a perennial ryegrass sward

Variable	Layer	May			June			October			Time		S.E.D.[a,b]	Effect[a]					
		21 days	28 days	35 days	21 days	28 days	35 days	21 days	28 days	35 days	Morn.	Even.		Month	age	time	month ×age	month ×time	age ×time
Tiller height (mm)		251	289	330	257	298	373	355	434	466	339	340	17.1	***	***	ns[c]	*	ns	ns
Sheath height (mm)		78	95	123	71	97	170	86	105	120	105	105	10.5	**	***	ns	***	ns	ns
Biomass (kg OM/ha)	0–5	1978	2218	2404	2123	2171	2413	1310	1299	1220	1891	1917	138.3	***	**	ns	*	0.10	ns
	5–10	1346	1530	1783	1086	1240	1387	667	690	738	1149	1177	97.6	***	***	ns	*	ns	ns
	10–15	730	907	1214	573	784	1025	508	552	572	747	779	65.3	***	***	ns	***	ns	ns
	+15	584	899	1542	523	903	1639	891	1337	1524	1053	1134	136.8	***	***	0.10	**	ns	ns
Bulk density (kg OM/ha/cm)	0–5	396	444	481	425	434	483	262	260	244	378	383	27.7	***	**	ns	*	0.10	ns
	5–10	269	306	357	217	248	277	133	138	148	230	235	19.6	***	***	ns	*	ns	ns
	10–15	146	182	243	115	157	205	102	111	115	149	156	13.1	***	***	ns	***	ns	ns
	+15	58	65	86	49	61	74	44	47	48	57	61	6.0	***	***	0.08	**	ns	ns
DM (g/kg fresh matter)	0–5	260	258	296	339	347	349	190	191	221	251	293	20.6	***	***	***	ns	**	ns
	5–10	207	213	239	212	224	235	131	129	165	179	211	14.8	***	***	***	ns	*	ns
	10–15	192	195	216	177	196	208	125	125	147	160	191	8.0	***	***	***	ns	**	ns
	+15	211	220	229	203	219	220	139	141	141	174	209	7.5	***	***	***	ns	*	ns
OM (g/kg DM)	0–5	813	819	839	720	769	780	769	762	818	777	799	28.1	***	**	*	ns	ns	ns
	5–10	863	883	900	826	889	898	875	872	884	870	883	15.5	ns	***	*	**	ns	ns
	10–15	874	892	907	885	909	923	880	870	880	887	895	7.1	***	***	**	***	ns	*
	+15	889	904	917	906	917	926	890	880	883	897	905	5.9	***	***	***	***	ns	*

[a] Standard error of the difference.
[b] Within a row, the less significant difference between months and regrowth ages is obtained on multiplying S.E.D. by the *t* value of 2.074.
[c] ns $p>0.10$.
[*] $p<0.05$; ** $p<0.01$; *** $p<0.001$.

The average biomass in layers 4, 3, 2 and 1 was, respectively, 1.1, 0.8, 1.2 and 1.9 t OM/ha ($p<0.001$, Table 4). Bulk density increased strongly from the top to the base of the sward, with 59, 153, 233 and 381 kg OM/ha/cm in layers 4, 3, 2 and 1, respectively ($p<0.001$).

On average, the range in biomass and bulk density among layers were much lower in October than in May and June (interaction layer×month: $p<0.001$ for both variables) (Table 2 and Fig. 1). In October, biomass and density were very low in the lower layers, while biomass in the upper layer showed less variation with month. Biomass increased with regrowth age in all layers, but much more in Layer 4 than in the other three layers, following the increase in height. On average, grass growth rate was 64, 24, 19 and 15 kg OM/ha per day in layers 4, 3, 2 and 1, respectively. However, the increase in biomass with regrowth age in the three lower layers was only significant in May and June (interaction layer×month×age: $p<0.05$). The increase in biomass with regrowth age in Layer 4 averaged 74 and 45 kg OM/ha per day in spring and autumn, respectively. On average, bulk density increased with age of regrowth in all layers, and much more in May and June than in October. The interaction layer×month×age was highly significant ($p<0.001$) for bulk density. In fact, the increase in bulk density with regrowth age in spring was the highest in Layer 3 (+6.6 kg OM/ha/cm per day) and lowest in Layer 4 (+1.9 kg OM/ha/cm per day), indicating that the greatest herbage mass accumulation per cm occurred in the intermediate layers.

Biomass and bulk density in Layer 4 both showed a tendency to higher values in the evening compared with the morning (+8%, $p<0.10$, Table 2). The time of day had no effect on either biomass or density in the three lowermost layers.

3.2. Dry matter and organic matter content

The average DM content in layers 4, 3, 2 and 1 was 191, 176, 195 and 272 g/kg fresh grass, respectively ($p<0.001$, Table 4). The DM content was highest in Layer 1 and lowest in Layer 3, whatever the month, regrowth age or time of day (Fig. 1). In all layers, the DM content was lower in October than in May and June, and differences in DM content between layers were more marked in June than in May and October due to a high DM content of the lower layer in June. The DM content increased slightly but significantly with ageing by 0.7–1.5 g/kg fresh grass per day according to the layer, and differences in DM content between layers were not fundamentally affected by ageing. The DM content was significantly higher in the evening than in the morning in all the layers, with a mean difference of 35 g/kg fresh grass (Fig. 2). The difference of DM content between morning and evening was greater in spring (44 g/kg) than in autumn (19 g/kg), with the interaction month×time of day being significant in all layers. The total amount of water present in the sward fell by 3.9 t/ha in the evening as compared with the morning, the loss being distributed uniformly between the different layers (about 1 t of water per layer).

On average, the OM content in layers 4, 3, 2 and 1 was, respectively, 901, 891, 877 and 788 g/kg DM ($p<0.001$). It decreased significantly and systematically from Layer 4 to Layer 1, whatever the month, regrowth age or time of day (Fig. 1). The OM content was particularly low in Layer 1 in June, due to soil contamination linked with the trampling of animals during the previous period of grazing that occurred under heavy rain. In the two

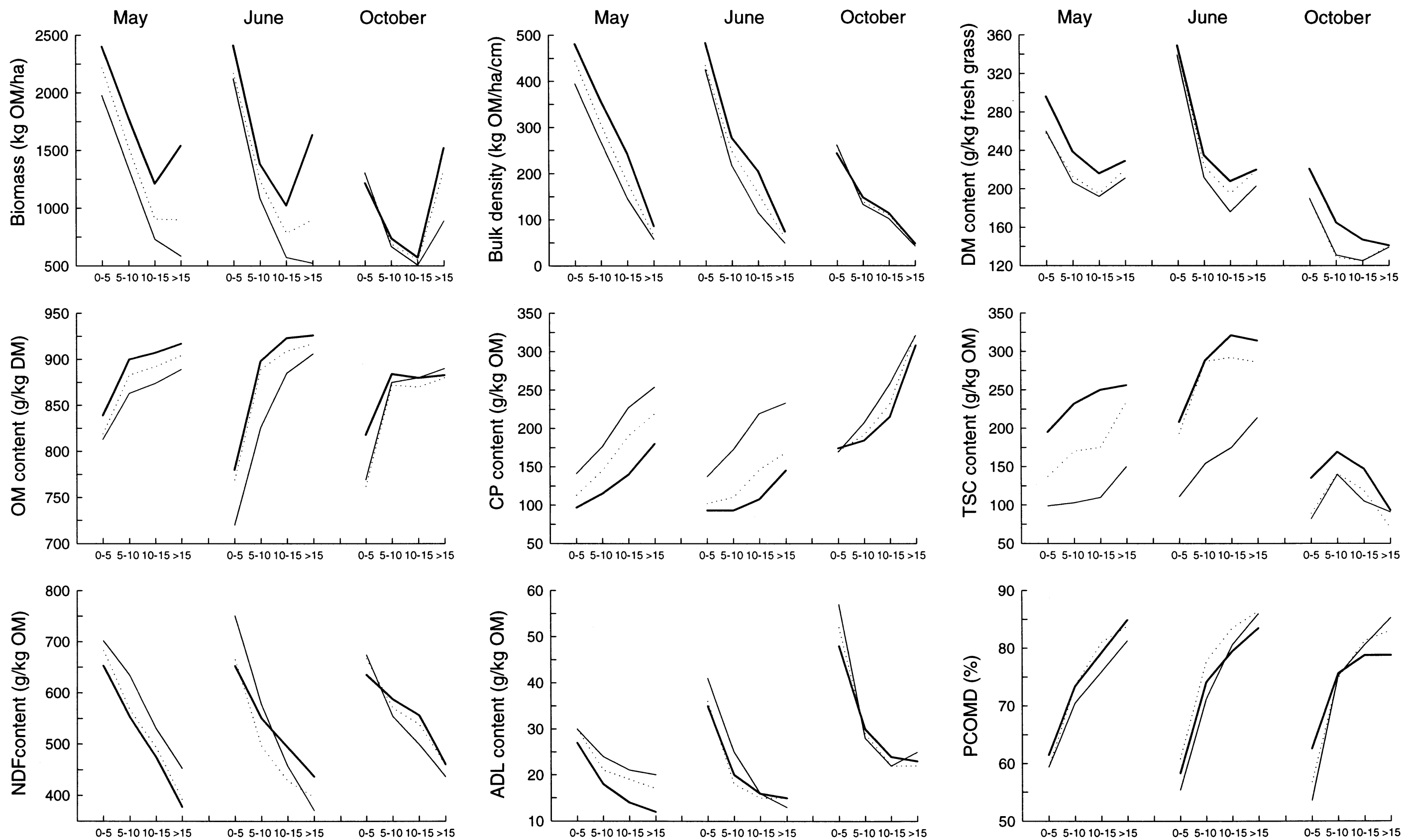

Fig. 1. Effect of month and regrowth age [(—), 21 days; (……), 28 days; (▬), 35 days] on the vertical distribution of biomass, bulk density, chemical composition and PCOMD in a perennial ryegrass sward.

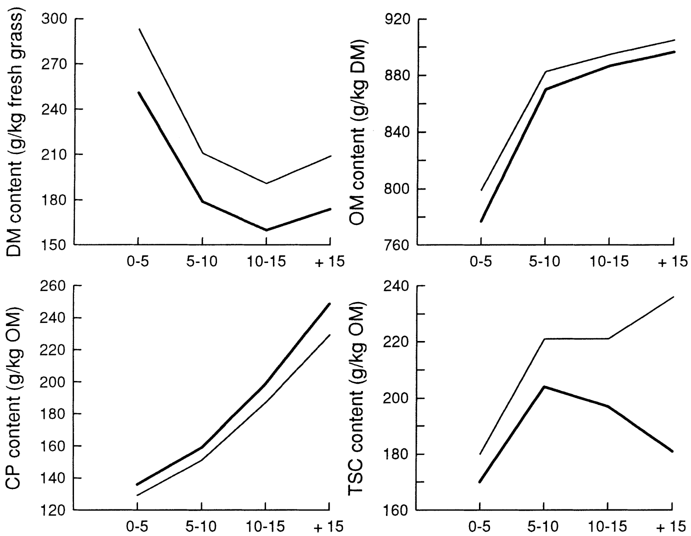

Fig. 2. Effect of time of day [(▬), morning; (—), evening] on the vertical distribution of DM, OM, CP and TSC contents in a perennial ryegrass sward.

upper layers, the OM content increased with regrowth age in May and June by an average of 2 g/kg DM per day, but did not show an increase in October (interaction layer×month×age: p<0.01) (Table 2). In all layers, the OM content was significantly higher (+13 g/kg DM) in the evening compared with the morning with no interaction between layer and time.

3.3. Crude protein and total soluble carbohydrates content

The average CP content in layers 4, 3, 2 and 1 was, respectively, 239, 193, 155 and 132 g/kg OM (p<0.001, Table 4). It decreased systematically from Layer 4 to Layer 1, for whatever month, regrowth age or time of day (Fig. 1). The CP contents were, on average, much higher in October than in May and June, and the differences between months were greater in the upper layers compared with the lower layers (interaction layer×month; p<0.001) (Table 3). In spring, the CP content fell sharply with regrowth age, this age-related effect being more marked in layers 3 and 4 (−6.1 g CP/kg OM per day) than in layers 1 and 2 (−3.7 g CP/kg OM per day) (interaction layer×month×age; p<0.01). The difference in CP content between Layer 4 and Layer 1 thus tended to decrease with ageing of the pasture. In autumn, ageing had very little effect on the CP content, whatever the layer. The CP content was significantly lower in the evening than in the morning in layers 3 and 4 (Table 3 and Fig. 2). The effect of time of day was far more pronounced in spring than in autumn with, on average, decreases of 22 and 4 g CP/kg OM above 10 cm, respectively (interaction layer×month×time of day: p<0.05).

The TSC content was, on average, 209, 209, 212 and 175 g/kg OM in layers 4, 3, 2 and 1, respectively (p<0.05). The vertical distribution of TSC content varied widely with the

Table 3
Effect of month, regrowth age and time of day on the vertical distribution of CP, NDF, ADL and TSC contents and on PCOMD in a perennial ryegrass sward

Variable	Layer	May			June			October			Time		S.E.D.[a,b]	Effect					
		21 days	28 days	35 days	21 days	28 days	35 days	21 days	28 days	35 days	Morning.	Evening		Month	Age	Time	Month ×age	Month ×time	Age ×time
CP (g/kg OM)	0–5	141	113	97	134	102	93	169	170	174	136	129	15.1	***	***	ns[c]	*	ns	ns
	5–10	176	145	115	172	110	93	207	191	184	159	151	14.5	***	***	ns	**	ns	ns
	10–15	227	190	140	219	147	108	258	232	215	199	187	13.8	***	***	*	***	*	ns
	+15	254	219	180	233	168	145	321	323	308	249	229	14.1	***	***	***	***	ns	0.09
TSC (g/kg OM)	0–5	122	167	232	153	251	266	106	116	165	170	180	13.7	***	***	ns	0.06	ns	ns
	5–10	120	192	258	186	323	321	160	161	191	204	221	14.7	***	***	0.08	*	ns	ns
	10–15	125	196	276	197	321	347	119	137	168	197	221	14.3	***	***	*	*	ns	ns
	+15	169	258	279	236	311	339	102	80	105	181	236	18.6	***	**	**	*	ns	ns
NDF (g/kg OM)	0–5	702	683	653	735	665	653	675	667	636	669	680	47.5	ns	*	ns	ns	ns	ns
	5–10	634	567	553	578	496	551	555	571	588	564	568	29.5	**	**	ns	**	ns	ns
	10–15	531	494	475	457	428	494	500	539	557	498	496	23.9	***	ns	ns	***	ns	0.07
	+15	453	392	378	371	396	437	437	459	461	433	408	25.9	***	ns	**	***	ns	ns
ADL (g/kg OM)	0–5	30	30	27	40	36	35	57	52	48	40	39	3.4	***	**	ns	ns	ns	ns
	5–10	24	21	18	25	18	20	28	29	30	24	24	3.0	***	*	ns	0.06	ns	ns
	10–15	21	19	14	16	15	16	22	22	24	18	19	2.2	***	ns	ns	**	ns	ns
	+15	20	17	12	13	15	15	25	23	23	18	18	3.2	***	ns	ns	*	ns	ns
PCOMD (%)	0–5	59.4	59.4	61.5	55.4	60.8	58.3	53.6	56.9	62.6	58.3	58.9	1.70	ns	*	ns	0.09	ns	ns
	5–10	70.6	73.4	73.4	71.3	77.7	74.2	75.2	74.9	75.6	73.4	74.6	0.96	*	*	0.07	*	ns	ns
	10–15	75.9	81.0	79.2	80.6	83.5	79.6	80.6	81.4	78.8	79.9	80.2	0.83	*	**	ns	*	ns	0.06
	+15	81.3	83.9	85.0	86.0	86.4	83.5	85.3	83.1	78.8	82.9	84.5	1.67	0.10	ns	ns	0.09	ns	ns

[a] Standard error of the difference.

[b] Within a row, the less significant difference between months and regrowth ages is obtained on multiplying S.E.D. by the *t* value of 2.074 (CP, NDF, ADL) or 2.776 (TSC, PCOMD).

[c] ns $p>0.10$.

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

month, regrowth age and time of day (Figs. 1 and 2). The TSC content was highest in layers 3 and 4 in May and June, but in Layer 2 in October (interaction layer×month: $p<0.05$). It increased strongly with regrowth age in all layers, this increase being the greatest in Layer 3 (+8.4 g/kg OM per day) and lowest in Layer 4 (5.1 g/kg OM per day) (interaction layer×age: $p<0.05$). Moreover, the increase in TSC content with regrowth age was much more pronounced in May and June (+7–11 g/kg OM per day according to the layer) compared with October (+0–4 g/kg OM per day according to the layer). The interaction month×age was significant for all layers. The TSC content was higher in the evening than in the morning for all layers, and the difference between morning and evening values was much greater in the upper than in the lower layers (Fig. 2). These differences averaged 56, 24, 17 and 10 g/kg OM for layers 4, 3, 2 and 1, respectively (interaction layer×time of day: $p<0.09$). The effect of time of day on TSC content was so important that this was highest in Layer 2 in the morning and in Layer 4 in the evening (Fig. 2). The total amount of TSC present in the sward (products of biomass and content) increased by 147 kg/ha between the morning and the evening, of which 75 kg was located in Layer 4.

The contents of TSC and CP showed a close negative correlation in each of the layers (R^2=0.69–0.95; n=18 per layer) (Fig. 3). The slope of the regression did not significantly vary with the layer and averaged −0.55 g CP/g TSC.

3.4. Neutral detergent fibre and acid detergent lignin content

The NDF and ADF contents were strongly correlated, irrespective of the layer, month, regrowth age or time of day. The following relationship was established from the whole

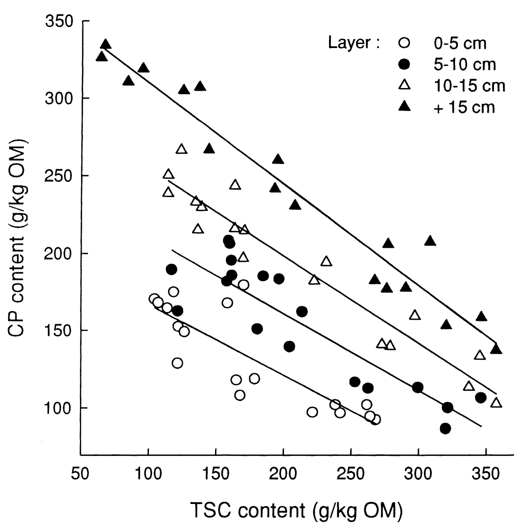

Fig. 3. Correlations for each layer between CP and TSC contents in a perennial ryegrass sward.

Table 4
Mean effect of layer number and their interactions with month, regrowth age and time of day on biomass, bulk density, chemical composition and PCOMD in a perennial ryegrass sward

Variable	Effect							Mean effect of layer		
	Layer	Layer ×month	Layer ×age	Layer ×time	Layer ×month ×age	Layer ×month ×time	Layer ×age ×time	Linear	Quadratic	Cubic
Biomass	***	***	***	ns[a]	*	ns	ns	***	***	***
Bulk density	***	***	***	ns	***	ns	ns	***	***	***
DM	***	***	***	**	ns	0.07	ns	***	***	***
OM	***	***	***	ns	**	0.09	ns	***	***	***
CP	***	***	***	**	**	*	ns	***	***	ns
NDF	***	***	***	*	**	ns	0.07	***	***	**
ADL	***	***	0.10	ns	**	ns	ns	***	***	***
TSC	*	*	*	0.09	ns	ns	ns	**	***	**
PCOMD	***	*	ns	ns	ns	ns	ns	***	***	***

[a] $p>0.10$.
* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

data set: ADF=0.481NDF+20.5; rsd=12.1; R^2=0.94; n=144. Consequently, ADF results will not be presented.

On average, the NDF content was 420, 497, 566 and 674 g/kg OM in layers 4, 3, 2 and 1, respectively ($p<0.001$, Table 4). It increased systematically and to a considerable extent from Layer 4 to Layer 1, whatever the month, regrowth age or time of day (Fig. 1). The range of NDF content between layers was higher in October than in May and June (interaction layer×month; $p<0.001$), with higher NDF contents only in the upper layers in October than in May and June (average of +50 g/kg OM in layers 3 and 4). The influence of regrowth age differed according to the layer and the month (Table 3). The NDF content decreased with regrowth age in layers 1 and 2 whatever the month, but showed little variation with regrowth age in layers 3 and 4. However, the NDF content in the two upper layers decreased with regrowth age in May, and increased in June and October (interaction layer×month×age: $p<0.01$). The NDF content of Layer 4 was significantly lower in the evening than in the morning by 25 g/kg OM on average.

The average ADL content was 18, 19, 24 and 39 g/kg OM in layers 4, 3, 2 and 1, respectively ($p<0.001$). It increased from Layer 4 to Layer 1 whatever the month, regrowth age or time of day, and was always much higher in Layer 1 than in the three other layers (Fig. 1). The ADL content was higher in October than in May and June for all layers. The range of ADL content between layers increased with advancing season, ADL content increasing much more with month of year in the lower layer than in the three upper layers (+23 and +8 g/kg OM between May and October, respectively, in Layer 1 and in layers 2, 3 and 4) (interaction layer×month; $p<0.001$). The ADL content variation with regrowth age differed between layers and between months (interaction layer×month×age; $p<0.01$). On average, the ADL content decreased significantly with ageing in layers 1 and 2, and did not vary with ageing in the two upper layers. However, ADL content also decreased with age in the upper layers in May as with NDF content (Table 3). Whatever the layer, the time of day never affected ADL content.

3.5. Organic matter digestibility from pepsin–cellulase (PCOMD)

The average PCOMD was 83.7, 80.0, 74.0 and 58.6% in layers 4, 3, 2 and 1, respectively (p<0.001, Table 4). It showed a strong and systematic decrease from the top to the base of the sward whatever the month, regrowth age or time of day (Fig. 1). The vertical distribution of PCOMD showed little variation with the month (Fig. 1). Although the PCOMD varied significantly with regrowth age in layers 1, 2 and 3, no clear trend might be recognized. As seen in the case of NDF content, this effect differed according to the layer and month. The PCOMD tended to increase with regrowth age in layers 1 and 2 in all months. For layers 3 and 4, PCOMD increased with regrowth age in May and decreased in June and October. The drop in digestibility at 35 days of regrowth during the autumn is linked to the senescence of the grass (onset of rotting clearly visible).

3.6. Prediction of diet composition

The data presented were used to develop expressions for predicting biomass, CP content, NDF content and PCOMD as a function of height, H (in cm), in the sward (Table 5):

$$\text{Bulk density (kg OM/ha/cm)} = a_1 - K_1 \times \ln\left(100\frac{H}{H_0}\right) \tag{1}$$

$$\text{CP content (g/kg OM)} = a_2 + \left[\frac{2K_2}{[1+\exp(-b(H-\text{ESH}))]}\right] \tag{2}$$

$$\text{NDF content (g/kg OM)} = a_3 + [K_3 \times \exp(-b \times H)] \tag{3}$$

$$\text{PCOMD (\%)} = a_4 + K_4 \times [1 - \exp(-b \times H)] \tag{4}$$

Table 5
Parameters for the prediction of the vertical distribution of biomass, chemical composition and PCOMD in a perennial ryegrass sward in spring and autumn (see equations in the text)

Variable	Season	n	A_0	B_0	A_{max}	B_{max}	b	S.D.[a]
Bulk density (kg OM/ha/cm)	Spring	24	733	1.401	172	−0.012	–	25.8
	Autumn	12	409	−1.084	85	0.006	–	11.3
CP content (g/kg OM)	Spring	24	128	−1.00	118	−2.37	0.299	13.9
	Autumn	12	0.78	3.06	202	−2.60	0.113	6.1
NDF content (g/kg OM)	Spring	24	283	2.28	691	−9.67	0.091	29.8
	Autumn	12	290	4.95	502	−7.76	0.087	11.9
PCOMD (%)	Spring	24	37.9	0.311	46.9	−0.282	0.155	1.97
	Autumn	12	−4.3	1.490	96.1	−1.821	0.239	0.82

[a] Standard deviation.

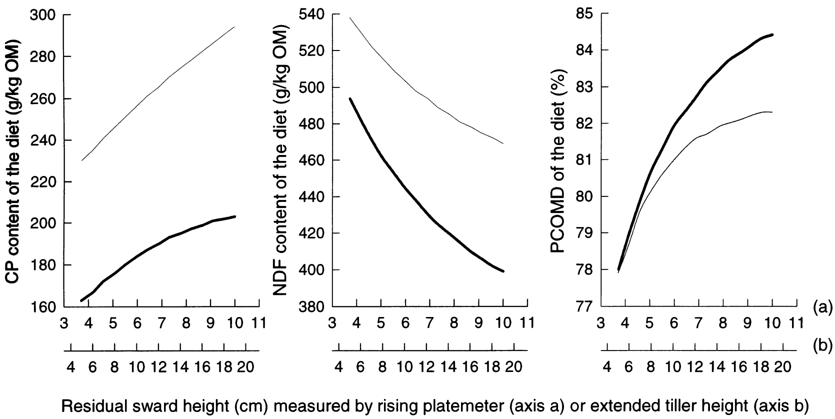

Fig. 4. Effect of the residual sward height on the predicted CP content, NDF content and PCOMD of the diet in a perennial ryegrass sward under strip-grazing management in spring (━━) and autumn (—).

where a_i=A_0+B_0×AGE (d), K_i=A_{max}+B_{max}×AGE (d), H_0 the maximum tiller height in the sward (cm)=1.3×mean extended tiller height (Wade, 1991), and ESH the mean extended sheath height in the sward (cm).

The parameters a_i (asymptotic value) and K_i (amplitude) were indexed on the regrowth age (AGE). Equations (1)–(4) were established for spring by pooling data from May and June (n=24), and for autumn by using data from October (n=12). The parameters of the equations (A_0, B_0, A_{max}, B_{max} and b) which minimize the residual variance for each season are given in Table 5. Integration of the biomass and chemical composition curves from the top of the sward (H_0) to a given residual height allows us to simulate the chemical composition of the selected grass.

Fig. 4 illustrates a simulation of the chemical composition of the selected grass as a function of the residual height for a perennial ryegrass sward at 28 days of regrowth and with a mean extended tiller height of 30 cm. As an example, a reduction of the residual height by about 2 cm (1 cm measured by rising plate-meter) around a value of 10 cm (6 cm measured by rising plate-meter) would lead to a variation of −8 g CP/kg OM, −1.0 unit in PCOMD (%) and +15 g NDF/kg OM for the overall diet in spring. The influence of residual height on the chemical composition of the ingested grass becomes even stronger as the residual height diminishes.

Fig. 5 shows a simulation of the variation of the chemical composition of ingested grass in the context of rotational grazing in spring down to a residual height of 8 cm (5 cm measured by rising plate-meter). In this simulation, we do not take account of grass growth during the period of grazing by the herd or any possible rapid changes in composition that might occur at the top of the sward after a defoliation. The CP content and PCOMD decrease mainly during the second half of the period of grazing of the paddock, while the CP content, PCOMD and NDF content at the end of paddock grazing represent 67, 88 and 142% of the initial contents at the beginning of paddock grazing, respectively.

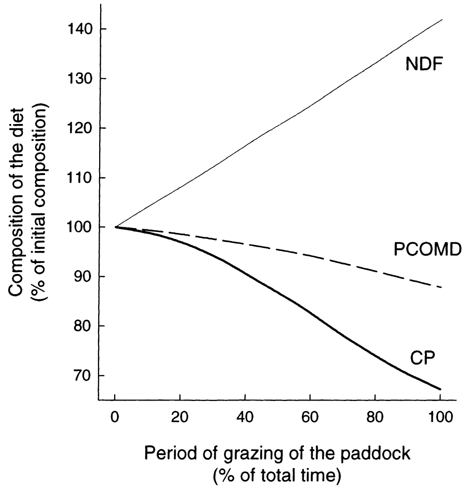

Fig. 5. Predicted CP content, NDF content and PCOMD of the diet (as a percentage of initial composition of the diet) during the grazing down process in a perennial ryegrass sward rotationally grazed to a residual sward height of 5 cm, measured by a rising platemeter (i.e. 8 cm of extended tiller height).

4. Discussion

4.1. Overall vertical distribution

The chemical composition of a rotationally grazed perennial ryegrass sward showed very large variations from the top to the base of the sward. The overall decrease in CP content and PCOMD, as well as the increase in DM, NDF and ADL contents with increasing depth in the sward, are all features that are compatible with results in the literature. In a fundamental way, these parameters reflect the proportion of blades, sheaths, stems and dead tissue in each of the layers (Wilkinson et al., 1970; Clark et al., 1974; Wilman et al., 1976; Johnston et al., 1993; Jarrige et al., 1995). The differences between layers were commonly more marked than the differences between months — or between regrowth ages — for a given layer or for the whole plant. Moreover, even if season and regrowth age affect the absolute values as well as the differences in content between layers, there was never any influence on the ranking of the layers. These results suggest that the relative proportions of the different morphological components within a given layer (blades, sheaths and stems) have an essential role in controlling the chemical composition of the layer concerned.

The DM content was shown to be systematically at a minimum in Layer 3 and not in the upper layer. In swards of *Cynodon dactylon*, Wilkinson et al. (1970) also observed that the intermediate layers have the highest water contents. These layers are probably the most enriched in leaf sheaths and their water content would be higher than those observed in blades even in full growth due to the role of sheaths in conducting sap (Jarrige et al., 1995).

4.2. Vertical distribution: effect of month

The experimental design does not allow a clear investigation of the effect of season on chemical composition, because the data set does not discriminate between the effects of the different months and fenced areas concerned. However, interactions between layer and month can be fully interpreted.

The vertical distribution of all chemical constituents varied with the month of sampling, and particularly with the season (May and June vs. October). However, the variability in the vertical range of constituent content between seasons resulted mainly from the marked differences in chemical composition between seasons. In particular, CP content increased and TSC content decreased in the autumn compared with the spring, which is in good agreement with previous reports (Waite and Boyd, 1953; Wilman et al., 1996). Strong increase in ADL content in the lower layer in October may reflect dead material accumulation after a complete season of grazing.

The vertical distribution of TSC content was greatly affected by month of the year, and the highest TSC contents were observed in the upper layers in spring. These results differ from those of Herrera et al. (1984), who observed the highest TSC contents close to the ground in their studies on *Cynodon dactylon*. Similarly, the majority of authors have shown that the sheaths and stems of grasses contain generally more total soluble carbohydrates than the blades, and particularly the long-term carbohydrate reserve fraction, i.e. fructanes (Waite and Boyd, 1953; Deinum and Dirven, 1975; Jarrige et al., 1995). In the absence of any marked water or N stress, the blades are generally poorer in fructanes. However, the blades may contain considerable amounts of mono- (glucose and fructose) as well as di- (saccharose) saccharides, especially at the end of the day (McIlroy, 1967). In spring, the overall increase of TSC content with height in the sward probably reflects strong photosynthetic activity and a high sugar/fructane ratio in the upper layers. In October, shorter day-length results in lower sugar synthesis, low sugar/fructane ratio in soluble carbohydrates, and very low TSC contents in the upper layers where fructanes are scarce (Jarrige et al., 1995).

4.3. Vertical distribution: effect of regrowth age

The vertical distribution of chemical composition was, on average, more affected by ageing than by season. Moreover, the effect of ageing on the vertical distribution of chemical constituents was important in spring, but not in autumn.

On average, the crude protein content decreased with ageing in spring, with values close to those reported by Wilman et al. (1976). This occurred in all the layers, indicating that nitrogen is diluted during growth in the leaf blades just as in the stems and sheaths. Mowat et al. (1965) and Wilman et al. (1976) have already shown that the nitrogen content of the leaf blades decreases with age to the same extent as in the sheaths and stems. This phenomenon is characteristic of grasses, while legumes show little variation of nitrogen content in leaves with ageing, in contrast to their stems and petioles (Gastal and Lemaire, 1997). In the present study, the decrease of crude protein content with age was even more marked in the upper layers than at the base of the sward, probably due to additional enrichment of sheaths and stems in the upper layers during growth. It is known that these organs are always poorer in nitrogen than the leaf blades (Wilman et al., 1976; Jarrige et al., 1995).

In spring, the TSC content increased twofold between 3 and 5 weeks in all the layers. Such an increase cannot be explained simply by changes in the leaf/stem ratio. Wilman (1980) also reports a doubling of total soluble carbohydrates content between 3.5 and 5.5 weeks of regrowth on ryegrass and fescue during the spring, while soluble carbohydrates storage was practically nil before 3 weeks and greatly reduced after 6 weeks of regrowth. This increase could result from a rise in photosynthetic activity following the increase of biomass and leaf area index (LAI) during the period of maximal growth. The highest increase in TSC content with ageing was observed in the intermediate layers, and probably reflects fructane accumulation in stem and sheaths (Waite and Boyd, 1953). The decrease in CP content with ageing in all layers may be linked directly to the increase in total soluble carbohydrates content mentioned above (Waite and Boyd, 1953; Mcllroy, 1967). The very close relationship found here between CP and total soluble carbohydrates clearly indicates an interdependence between the metabolic activities of carbohydrates and nitrogen (Ferrario et al., 1997).

The effect of ageing on cell-wall content and PCOMD differed according to the layer. In the upper layers, the cell-wall constituent contents and PCOMD values were generally not affected by ageing, which is in agreement with studies carried out on grass species at the leafy stage (Corbett et al., 1963; Deinum and Dirven, 1975; Wilman, 1975; Demarquilly and Andrieu, 1988). The effect of ageing on lignification and digestibility is far less marked before the stem elongation stage than afterwards, due to a slowing down in the change of the leaf/stem ratio. This is because the decrease in digestibility with regrowth age is much less for the leaves than for the stems (Terry and Tilley, 1964; Mowat et al., 1965). In the lower layers, cell-wall content generally decreased and PCOMD increased with ageing. The reason for this is not clear and similar results are not described in the literature. In fact, few studies have been carried out on the chemical composition of grass in the first cm above ground level. Clark et al. (1974) reported a significant decrease in in vitro digestibility with ageing in the lowermost layer (3–10 cm) of a mixed sward. However, in the experiment of Clark et al. (1974), ageing was mixed with a decreasing proportion of clover, which may partly explain the decrease of in vitro digestibility. In our experiment, biomass accumulation with ageing also occurred in the lower layers, so an increased proportion of new material might have diluted the dead and lignified material. Even if this phenomenon could occur during the leafy stage, it probably does not take place at higher ages of regrowth when senescence is more active, resulting in the accumulation of dead and lignified material.

4.4. Vertical distribution: effect of time of day

The time of day for sampling had a marked effect on a large number of chemical constituents, particularly in the upper layers of the sward which represent the main site for photosynthesis and gas exchange with the atmosphere.

Active processes occurring between morning and evening mainly involve loss of water and gain in soluble carbohydrates. The increase in DM content in the evening may result from a loss of surface water and from a negative absorption–transpiration budget during most of the diurnal phase (Cruiziat, 1997). The difference in total soluble carbohydrates contents between morning and evening observed here is fully compatible with the nycthemeral variation described in the literature (Waite and Boyd, 1953; Holt and Hilst, 1969; Lechtenberg et al., 1972). In all the studied species, the total soluble carbohydrates content increased from morning to the evening by the accumulation of photosynthesized simple sugars (especially saccharose), and then fell during the night — in the absence of photosynthesis — by respiration, protein synthesis and export towards storage organs. In this way, Lechtenberg et al. (1972) have shown on *Festuca arundinacea* that 30% of the soluble carbohydrates accumulated up to 18.00 h disappears by 21.00 h and 63% by 24.00 h. The present results show that this process of consumption is maximal in the blade-rich upper layers of the sward. The morning/evening variations of the total soluble carbohydrates content were less pronounced in the lower layers of the sward, probably because of the lower proportion of blades and the reduction in photosynthesis due to increased shade.

The contents in other chemical constituents probably varied in a passive manner between morning and evening by concentration (OM, digestible OM) or dilution (CP, NDF) within the dry matter following the increase in the amount of soluble carbohydrates. In fact, the amounts of CP and NDF (products of biomass and content) in each layer were the same in the morning as in the evening while only the amount of total soluble carbohydrates showed a strong increase.

The present results also suggest that, under strip-grazing management, making the paddock available to grazing animals in the evening, rather than in the morning, will increase the total amount of total soluble carbohydrates ingested. The time of day used for access to the paddock will have no major effect on the other chemical constituents of the diet. Orr et al. (1998) studied the practical consequences of the time of day for allowing access to a paddock, and observed a tendency to increased milk production during the spring provided access is given in the evening, rather than in the morning.

5. Conclusion

This study has revealed that variations in the chemical composition of perennial ryegrass associated with height in the sward are predominant over — and frequently more marked — than variations in the composition of the whole plant linked to season or regrowth age in the vegetative stage. For a given chemical constituent, the ranking between layers remained unaffected by season, regrowth age or time of day (except for TSC content). On the other hand, the differences in contents between layers were sometimes strongly dependent on season (bulk density, CP, TSC), regrowth age (CP) or time of day (TSC). The vertical gradients in NDF and PCOMD were relatively insensitive to season or regrowth age. These data make it possible to predict the variation of chemical composition of grass selected by ruminants during the grazing-down process.

Acknowledgements

The authors would like to thank F. Gastal for helpful comments during the preparation of the manuscript and M.S.N. Carpenter for translation of the paper into English and later revision work.

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