Journal of Agricultural Science, Cambridge (1998), **131**, 51–58. *Printed in the United Kingdom*

In vitro digestibility and neutral detergent fibre and lignin contents of plant parts of nine forage species

D. WILMAN* AND P. REZVANI MOGHADDAM†

Welsh Institute of Rural Studies, University of Wales, Aberystwyth, Ceredigion, SY23 3AL, UK

(*Revised MS received 30 January 1998*)

SUMMARY

In order to understand better some of the reasons for differences between forage plant species in digestibility, different parts of nine plant species in either milled or chopped (1 cm lengths) form were examined for *in vitro* digestibility and in milled form for neutral detergent fibre and lignin. The nine species were: *Trifolium repens* L., *Medicago sativa* L., *Desmodium intortum* (Mill.) Urb., *Brassica napus* L., *Lolium perenne* L., *Festuca arundinacea* Schreb., *Chloris gayana* Kunth, *Cenchrus ciliaris* L. and *Zea mays* L. In each case early harvesting was compared with later harvesting in each of two years. The plants were grown in spring–summer in a heated glasshouse.

The forage at the early harvest was, on average, 1–4% units more digestible *in vitro* than that at the later harvest and generally slightly lower in lignin and neutral detergent fibre content. However, the stems of *Z. mays* were higher in neutral detergent fibre at the early than at the later harvest.

The leaf sheaths of *L. perenne* and *F. arundinacea* were more digestible than the leaf blades. *L. perenne* was more digestible than *F. arundinacea* in both sheaths and blades. The sheaths and blades of *C. gayana* and *C. ciliaris* were less digestible and had a higher neutral detergent fibre content than those of *L. perenne* and *F. arundinacea*. The leaf blades, excluding the midribs, and the stems and leaf sheaths of *Z. mays* were all rather high in digestibility when milled and moderately low in neutral detergent fibre and lignin; the leaf blade midribs of *Z. mays* were less digestible and higher in neutral detergent fibre than the stems and similar to the stems in lignin content. The leaflets of *T. repens* had an appreciably lower neutral detergent fibre content than the stolons and petioles and a rather lower lignin content in dry matter and yet were, if anything, less digestible than the stolons and petioles. The stolons of *T. repens* were much more digestible than the stems of *M. sativa* and *D. intortum*. The digestibility of *D. intortum* was low in all the plant parts examined, leaflets, petioles and stems. In both *D. intortum* and *B. napus*, the leaflets or leaf blades were much lower than the stems in neutral detergent fibre and lignin and yet they were no more digestible than the stems when milled.

The digestibility of chopped leaflets and leaf blades was similar to that of milled leaflets and leaf blades, but chopping rather than milling reduced the digestibility of stems (particularly of those of *Z. mays*), petioles, the leaf blade midribs of *Z. mays*, and, to some extent, leaf sheaths.

INTRODUCTION

It is important to understand more fully the reasons for differences in digestibility between forages in order that improvements can be made in husbandry and plant breeding. Because of the wide range of plant material used for forage, it is useful to compare contrasting plant types, monocotyledonous and dicotyledonous, temperate and tropical, when they are grown in identical conditions, and also to divide the plant material into the main component parts, which are often very different in morphology and anatomy. In their review of the digestibility of plant parts, Hacker & Minson (1981) were able to refer to many forage species, but in most cases information on the various species was from different sources and different environments, making it difficult to compare species satisfactorily.

In the present study, we have compared nine forage species, covering a wide range of plant types and grown in identical conditions, and we have divided the harvested forage into component parts. Inform-

* To whom all correspondence should be addressed. Email: ddw@aber.ac.uk

† Present address: Department of Agronomy, Faculty of Agriculture, Ferdowsi University, PO Box 91775–1163, Mashhad, Iran.

ation is already available on the morphology and vascular structure of plant parts from the same experiment (Wilman *et al*. 1996*a*), which is useful when interpreting the digestibility results. Wilman *et al*. (1996*a*) also recorded the *in vitro* digestibility of each forage (not divided into plant parts), the neutral detergent fibre (NDF) content (as an estimate of the proportion of cell wall) and the proportion of different plant parts. In the present study we have recorded the *in vitro* digestibility and NDF content of the plant parts.

A conventional test for *in vitro* digestibility, such as that of Tilley & Terry (1963), which was used by Wilman *et al*. (1996*a*) and in the present study, involves grinding dried samples to produce particles small enough to pass through a fine sieve of 0·8–1·0 mm; the grinding increases the proportion of cell wall which is immediately accessible to the microbes in the rumen fluid. *In vivo*, many of the particles to be digested in the rumen are larger than this, so that the access of microbes to cell wall is more restricted than for milled material, particularly in those parts of particles which are made up of thick-walled cells tightly packed together, as noted by Wilson & Mertens (1995). In order to indicate the extent to which more restricted access to cell wall may reduce the *in vitro* digestibility of different plant parts of different species, we have also recorded the digestibility of 1-cm pieces of plant tissue and compared this with the digestibility of milled tissue.

The presence of lignin in cell wall can restrict digestion, at least in cell walls in which the lignin concentration is very high, as commonly found in the xylem of forage legumes (Wilson & Mertens 1995). For this reason, we also measured the concentration of lignin in dry matter (DM) and in NDF in different plant parts of the nine species studied.

MATERIALS AND METHODS

The experimental treatments comprised all combinations of nine plant species and early *v*. later harvesting. A randomized block design was used, with two blocks. The nine species were: *Trifolium repens* L., *Medicago sativa* L., *Desmodium intortum* (Mill.) Urb., *Brassica napus* L., *Lolium perenne* L., *Festuca arundinacea* Schreb., *Chloris gayana* Kunth, *Cenchrus ciliaris* L. and *Zea mays* L.

As far as possible, the samples for analysis were taken from the experiment described by Wilman *et al*. (1996*a*). The plants in half the pots were harvested early, on 25–26 June 1991 and 22–25 June 1992, except that the three leguminous species were harvested on 23–24 July in 1991 (because of slower development). The plants in the remaining pots were harvested later (i.e. at a more mature stage), on 23–24 July 1991 and 22–25 July 1992, except that the three legumes were harvested on 20 August in 1991. The seed was sown on 25 April 1991; the annual species (*B. napus* and *Z. mays*) were re-sown on 23 April 1992 and the perennial species were cut back on the same date. Where there was insufficient sample for all the analyses, the samples were made up to the required weight by adding material grown in 1994 and 1995, as far as possible in identical conditions to those of 1991–92. In 1994, the seeds were sown on 24 April, the early harvest was on 28 June and the later harvest on 25 July, except that the harvest dates for the three legumes were 25 July and 23 August, respectively. In 1995, the annual species were re-sown and the perennial species cut back on 24 April, the early harvest for all species was on 19 June and the later harvest on 20 July. The numbers of days and the mean minimum and maximum temperatures between the date of sowing or cutting back and the dates of harvesting in each of the four years are shown in Table 1. The growth stage of each species at each harvest is indicated in Table 2 by the proportions of stem and leaf. There was no stem development in *F. arundinacea* and very little in *L. perenne*; in the remaining species, particularly *C. gayana*, the proportion of stem was rather higher at the later than at the early harvest.

The plants were grown in potting mix in 25-cm pots in a heated glasshouse. John Innes fertilizer and ground limestone were included in the potting mix at a rate, in each case, of 5.7 g/kg. Additional nutrients and potting mix were added as required; all pots were treated the same except that maize had more nutrients than the other species because of its greater growth potential. The pots were watered daily to keep the soil moist.

At each harvest the plants were cut *c*. 2 cm above the soil surface and herbage stored in a freezer at −16 °C. Subsamples for the present analyses were defrosted and separated into plant parts to provide leaflets, petioles and stems for analysis in the case of the legumes and *B. napus*, and leaf blades, leaf sheaths and stems in the case of the grasses; dead material and inflorescences were excluded. Because the subsamples were small, material from the two blocks was bulked. For statistical analysis, year 1 (1991, supplemented where necessary with material from 1994) was regarded as the first replicate and year 2 (1992, supplemented where necessary with material from 1995) was regarded as the second replicate and the years × treatments interaction was used as the error. In *Z. mays*, leaf blade midribs were analysed separately from the rest of the blade. In *M. sativa*, there was insufficient petiole for analysis and in *L. perenne* and *F. arundinacea* there was insufficient stem (Wilman *et al*. 1996*a*).

In vitro DM digestibility was determined by the two-stage technique of Tilley & Terry (1963), using rumen fluid from sheep. Digestibility was determined

Table 1. *Periods between sowing or cutting and early and later harvesting and mean daily minimum and maximum temperatures*

	Species	
	Trifolium repens *Medicago sativa* *Desmodium intortum*	*Brassica napus* *Lolium perenne* *Festuca arundinacea* *Chloris gayana* *Cenchrus ciliaris* *Zea mays*
Period between sowing or cutting and harvesting (days)		
Early harvesting		
1991	89	61
1992	61	61
1994	92	65
1995	56	56
Later harvesting		
1991	117	89
1992	91	91
1994	121	92
1995	87	87
Mean daily temperatures between sowing or cutting and harvesting (°C, minimum/maximum)		
Early harvesting		
1991	15/31	15/31
1992	14/31	14/31
1994	15/33	16/32
1995	15/35	15/35
Later harvesting		
1991	15/31	15/31
1992	14/31	14/31
1994	15/33	15/33
1995	16/35	16/35

Table 2. *Proportions of leaf and stem* (*% in dry matter*) *with early and later harvesting*, *means of 1991 and 1992*

	Early harvesting		Later harvesting	
Species	Green leaflet or leaf blade	Green stem	Green leaflet or leaf blade	Green stem
Trifolium repens	38	16	22	21
Medicago sativa	38	53	31	57
Desmodium intortum	43	44	41	45
Brassica napus	50	20	37	27
Lolium perenne	62	2	51	5
Festuca arundinacea	69	0	71	0
Chloris gayana	66	12	42	32
Cenchrus ciliaris	36	40	23	52
Zea mays	32	37	27	48
S.E.	3·4	3·7	3·4	3·7
Mean	48	25	38	32

in two sets of subsamples; one set was oven-dried at 85 °C for 24 h and milled through a 1 mm screen in the conventional way; the other set was chopped when unfrozen into 1 × 1 cm pieces in the case of large leaves, 1 cm lengths in the case of narrow leaves, petioles and stems, and left intact in the case of small leaflets. The latter set was not dried before determining digestibility, but subsamples were taken to determine

DM content and the same weight of DM (0·5 g per sample) was used as for the milled samples. All 100 samples from year 1 (50 milled and 50 chopped) were analysed in one week and all 100 samples from year 2 the following week.

Neutral detergent fibre (NDF), as an estimate of the proportion of cell wall, was determined by the Van Soest & Wine (1967) procedure, using 0·5 g freeze-dried herbage per sample and correcting to an oven-dry basis. NDF was not corrected for nitrogen, on the basis that freeze-drying (as distinct from oven-drying) would not denature the protein within the cells (Jones & Bailey 1972; Jones & Moseley 1993). A further 0·5 g of freeze-dried herbage per sample was boiled in acid detergent solution (Van Soest 1963) and lignin was determined as the loss in weight of the acid detergent residue when treated with potassium permanganate solution (Van Soest & Wine 1968).

RESULTS

In vitro *digestibility*

The forage at the early harvest was, on average, 1–4 % units more digestible *in vitro* than that at the later harvest in the plant parts which were recorded (Table 3). However, leaflets of *T. repens* and *M. sativa* were at least as digestible at the later as at the earlier harvest. The digestibility of chopped leaflets and leaf blades was similar to that of milled leaflets and leaf blades, but chopping rather than milling reduced the digestibility of stems, petioles, the leaf blade midribs of *Z. mays* and, to some extent, leaf sheaths. The reduction in digestibility due to chopping rather than milling was particularly marked in the case of *Z. mays* stems and, to a lesser extent, *C. ciliaris* stems.

The most digestible plant parts examined were the petioles of *B. napus*; the leaf blades and stems of this species were also highly digestible (Table 3). Other plant parts which were highly digestible were the leaf sheaths and leaf blades of *L. perenne* and *Z. mays* and the stems (i.e. stolons) of *T. repens*. It is of interest that the leaf sheaths of *L. perenne* and *F. arundinacea* were more digestible than the leaf blades and that the stems of *T. repens* were more digestible than the leaflets. The least digestible species was *D. intortum*, with a DM digestibility < 60 % except in the chopped leaflets at the early harvest. Other plant parts which were < 60 % digestible were the stems of *M. sativa* and the chopped stems of *C. gayana*, *C. ciliaris* and *Z. mays*.

The *T. repens* and *M. sativa* leaflets were similar in digestibility (Table 3). The leaf blades and leaf sheaths of *L. perenne* were more digestible than those of *F. arundinacea*. Differences in digestibility between *C. gayana* and *C. ciliaris* in the plant parts examined were generally not large enough to be statistically significant, but both these species were less digestible than *Z. mays*, except in the chopped stems.

Neutral detergent fibre

The NDF content of leaflets and leaf blades tended to be slightly higher at the later than at the earlier harvest, except in *T. repens*, *M. sativa* and *Z. mays* (Table 4). The NDF content of leaf sheaths also tended to be slightly higher at the later than at the earlier harvest, except in *L. perenne*. The NDF content of petioles and stems was, on average, not affected by time of harvest, and the only statistically significant effect of time of harvest was in *Z. mays* stems, in which NDF was reduced by delaying the harvest.

The plant parts with the lowest NDF content were the leaf blades of *B. napus*, followed by the leaflets of *M. sativa* and *T. repens*, followed by the petioles of *B. napus* (Table 4). The plant parts with the highest NDF content were the stems, leaf sheaths and leaf blades (particularly the stems) of *C. gayana* and *C. ciliaris*. In *L. perenne* the NDF content of the leaf sheaths was similar to that of the leaf blades; in *F. arundinacea* the leaf blades in particular had a higher NDF content than those of *L. perenne*. In *T. repens* the NDF content of the stems was similar to that of the petioles and much higher than that of the leaflets. The NDF content of *D. intortum* leaflets and petioles was much higher than that of *T. repens* leaflets and petioles and the NDF content of *D. intortum* stems was a little higher than that of *M. sativa* stems. The NDF content of *Z. mays* stems was intermediate between that of *T. repens* and *B. napus* stems, on the one hand, and that of *M. sativa* and *D. intortum* stems, on the other hand.

Lignin content

The percentage of lignin in the DM of all plant parts tended to be higher at the later than at the early harvest, but there were several exceptions, in particular the stems and petioles of *B. napus* and the stems of *M. sativa* (Table 4). The plant parts with the lowest percentage of lignin in DM were the leaf sheaths and leaf blades of *L. perenne*, the leaflets of *M. sativa* and the leaf blades (excluding midribs) of *Z. mays*. The plant parts with the highest lignin content in DM were the stems of *D. intortum* and *M. sativa*; the petioles of *D. intortum*, and the stems of *C. ciliaris*, *C. gayana* and *B. napus*, were also high in lignin. *D. intortum* leaflets were higher in lignin than the *T. repens* and *M. sativa* leaflets and also higher than the leaf blades of most of the grass species. The leaf sheaths of *C. gayana* and *C. ciliaris* were higher in lignin than the sheaths of the temperate grasses and *Z. mays*. The lignin content of *Z. mays* stems was similar, on average, to that of *Z. mays* leaf blade midribs and *T. repens* stems and lower than that of *B. napus* stems.

The percentage of lignin in NDF was lowest in the leaf blades of the grasses and the leaf sheaths of *L.*

Table 3. *In vitro dry matter digestibility* (%) *of milled and chopped plant parts of nine forage species, means of 2 years*

Plant part and species	Milled			Chopped		
	Early harvest	Later harvest	Mean	Early harvest	Later harvest	Mean
Leaflets or leaf blades						
Trifolium repens	68·4	69·7	69·1	70·5	70·8	70·7
Medicago sativa	70·8	70·8	70·8	71·5	71·8	71·6
Desmodium intortum	56·7	54·2	55·4	62·2	58·4	60·3
Brassica napus	76·3	73·4	74·8	80·6	74·0	77·3
Lolium perenne	74·1	72·5	73·3	72·7	75·1	73·9
Festuca arundinacea	69·1	66·4	67·7	65·6	65·5	65·6
Chloris gayana	68·4	64·8	66·6	69·7	59·8	64·7
Cenchrus ciliaris	72·7	67·5	70·1	73·1	66·6	69·9
*Zea mays**	76·0	71·9	74·0	80·0	68·7	74·4
S.E. (17 D.F.)	2·05	2·05	1·45	2·42	2·42	1·71
Mean	70·3	67·9	69·1	71·8	67·9	69·8
Leaf blade midribs						
Zea mays	64·2	62·9	63·6	54·3	52·1	53·2
Petioles						
Trifolium repens	72·5	72·1	72·3	70·9	67·3	69·1
Desmodium intortum	57·2	56·3	56·7	52·2	49·9	51·0
Brassica napus	84·9	84·1	84·5	84·4	82·3	83·4
S.E. (5 D.F.)	2·37	2·37	1·68	4·15	4·15	2·93
Mean	71·5	70·8	71·2	69·2	66·5	67·8
Leaf sheaths						
Lolium perenne	78·0	78·3	78·2	78·7	73·2	75·9
Festuca arundinacea	75·5	72·1	73·8	71·2	71·2	71·2
Chloris gayana	65·5	62·8	64·1	68·7	59·8	64·2
Cenchrus ciliaris	69·8	65·5	67·7	64·0	62·5	63·3
Zea mays	73·4	72·0	72·7	74·1	70·3	72·2
S.E. (9 D.F.)	1·14	1·14	0·81	2·84	2·84	2·00
Mean	72·4	70·1	71·3	71·3	67·4	69·4
Stems						
Trifolium repens	75·5	75·5	75·5	73·1	71·5	72·3
Medicago sativa	56·6	56·1	56·4	51·0	52·3	51·6
Desmodium intortum	53·8	55·0	54·4	50·7	46·1	48·4
Brassica napus	72·4	77·5	75·0	73·8	68·1	71·0
Chloris gayana	66·2	59·5	62·9	57·0	54·7	55·8
Cenchrus ciliaris	62·5	55·5	59·0	49·9	43·3	46·6
Zea mays	72·8	67·5	70·2	43·5	38·9	41·2
S.E. (13 D.F.)	3·14	3·14	2·22	5·88	5·88	4·16
Mean	65·7	63·8	64·8	57·0	53·5	55·3

* Excluding midribs.

perenne, *F. arundinacea* and *Z. mays* (Table 4). The plant parts with the highest percentage of lignin in NDF were the stems of *M. sativa*, *D. intortum* and *B. napus* and the petioles of *D. intortum*. The leaflets or leaf blades with the highest percentage of lignin in NDF were those of *D. intortum*, *B. napus* and *T. repens*. The stems with the lowest percentage of lignin in NDF were those of *Z. mays*.

Relationships

In the grass plant parts (including those of *Z. mays*) there was a strong negative correlation between digestibility and both NDF and lignin; the correlation coefficient (r) between the digestibility of milled material and NDF was $-0{\cdot}89$ ($n = 14$; $P < 0{\cdot}001$), taking the means of 2 years and of earlier and later harvesting; the equivalent correlation between digestibility and lignin in DM was $-0{\cdot}87$ ($P < 0{\cdot}001$) and that between NDF and lignin in DM was $+0{\cdot}85$ ($P < 0{\cdot}001$). For the dicotyledonous plant parts, the relationship between digestibility and NDF and lignin was less clear, but there was a strong positive correlation between NDF and lignin in DM ($r = +0{\cdot}98$; $n = 11$; $P < 0{\cdot}001$).

Table 4. *Neutral detergent fibre and lignin content of plant parts of nine forage species, means of 2 years*

	Neutral detergent fibre (% in dry matter)			Lignin (% in dry matter)			Lignin (% in neutral detergent fibre)
Plant part and species	Early harvest	Later harvest	Mean	Early harvest	Later harvest	Mean	Mean of both harvests
Leaflets or leaf blades							
Trifolium repens	24·0	21·8	22·9	2·5	2·5	2·5	11·1
Medicago sativa	22·1	21·5	21·8	1·6	1·9	1·8	8·2
Desmodium intortum	32·7	36·2	34·4	3·8	4·7	4·2	12·2
Brassica napus	16·1	16·5	16·3	1·8	2·1	2·0	12·0
Lolium perenne	48·9	51·6	50·3	1·4	2·0	1·7	3·4
Festuca arundinacea	56·3	61·0	58·6	2·2	2·5	2·3	4·0
Chloris gayana	67·8	73·0	70·4	3·0	3·5	3·3	4·6
Cenchrus ciliaris	64·6	68·7	66·7	1·9	2·8	2·3	3·5
*Zea mays**	58·9	55·9	57·4	1·6	1·9	1·8	3·0
S.E. (17 D.F.)	1·65	1·65	1·16	0·55	0·55	0·39	1·01
Mean	43·5	45·1	44·3	2·2	2·7	2·4	6·9
Leaf blade midribs							
Zea mays	71·2	73·4	72·3	4·4	4·9	4·6	6·4
Petioles							
Trifolium repens	38·8	38·7	38·7	4·9	5·2	5·0	13·0
Desmodium intortum	49·3	51·0	50·2	8·0	8·7	8·3	16·6
Brassica napus	28·2	26·7	27·5	3·4	2·9	3·2	11·5
S.E. (5 D.F.)	1·25	1·25	0·89	0·56	0·56	0·40	1·08
Mean	38·8	38·8	38·8	5·4	5·6	5·5	13·7
Leaf sheaths							
Lolium perenne	51·0	50·1	50·5	1·5	1·5	1·5	3·0
Festuca arundinacea	53·9	54·7	54·3	2·3	2·6	2·4	4·5
Chloris gayana	70·5	73·4	71·9	4·4	5·3	4·8	6·7
Cenchrus ciliaris	73·1	75·0	74·0	4·1	5·1	4·6	6·2
Zea mays	60·9	65·0	63·0	2·7	3·1	2·9	4·5
S.E. (9 D.F.)	2·03	2·03	1·43	0·28	0·28	0·20	0·28
Mean	61·9	63·6	62·7	3·0	3·5	3·2	5·0
Stems							
Trifolium repens	37·4	39·7	38·5	4·3	4·2	4·2	11·0
Medicago sativa	60·3	61·3	60·8	11·0	10·5	10·8	17·7
Desmodium intortum	65·5	64·1	64·8	10·7	11·4	11·1	17·1
Brassica napus	43·8	39·5	41·6	8·5	6·3	7·4	17·7
Chloris gayana	77·3	81·3	79·3	7·3	8·0	7·6	9·6
Cenchrus ciliaris	79·7	82·7	81·2	8·1	8·5	8·3	10·2
Zea mays	57·4	50·2	53·8	2·6	5·1	3·9	7·3
S.E. (13 D.F.)	1·61	1·61	1·14	0·66	0·66	0·47	0·79
Mean	60·2	59·8	60·0	7·5	7·7	7·6	12·9

* Excluding midribs.

DISCUSSION

The rather higher *in vitro* digestibility of leaf sheaths than leaf blades in *Lolium perenne* and *Festuca arundinacea* was a little unexpected. Contributory reasons may have been the greater distance between adjacent veins in the sheaths than in the blades in *L. perenne* (Wilman *et al.* 1996*a*) and a lower NDF content in sheaths than blades in *F. arundinacea* (Table 4). As noted by Wilson (1976*a*, *b*), grass leaf sheaths may be developmentally younger than their blades and there may be only weak cuticle development on sheath tissue which is fully enclosed within older sheaths. On the other hand, the majority of the sheath tissue (of *Panicum maximum*) sampled by Wilson (1976*a*) was less digestible *in vitro* than equivalent leaf blade tissue. Terry & Tilley (1964) recorded lower *in vitro* DM digestibility in sheaths than blades in *L. perenne* and *F. arundinacea*, except in crops harvested at a very early stage of maturity. Wilman *et al.* (1996*b*) recorded rather lower *in vitro* true DM digestibility in sheaths than blades of

vegetative tillers of *L. perenne* and *Lolium multiflorum*. In the present study, the lower digestibility of *F. arundinacea* than of *L. perenne* (Wilman *et al*. 1996*a*) was evident in both blades and sheaths. It seemed from the comparison of chopped with milled forage that rumen microbes can reach the inner cells of the blades and sheaths of these two grasses without too much difficulty, even when presented with fairly large pieces of blade or sheath; mature stems, however, might include more cells which are difficult to reach, as was evidently the case with the stems of *C. gayana* and *C. ciliaris* and the stems and leaf blade midribs of *Z. mays* in the present study. It should be noted when comparing the *in vitro* digestibility of chopped with milled forage in the present experiment that the chopped material was incubated fresh whereas the milled material had been oven-dried before milling; it seems likely, however, that the oven drying (for 24 h at 85 °C) would not have significantly affected *in vitro* digestibility (Tilley & Terry 1963).

The lower whole crop digestibility and higher NDF content of *C. gayana* and *C. ciliaris* than of *L. perenne* and *F. arundinacea*, noted by Wilman *et al*. (1996*a*), was evident in the leaf sheaths and, in the case of NDF, in the leaf blades (Tables 3 and 4). The greater proportion of the widths of the sheaths and blades occupied by veins in *C. gayana* and *C. ciliaris* than in *L. perenne* and *F. arundinacea* (Wilman *et al*. 1996*a*) would contribute to the higher fibre content and lower digestibility. The higher lignin content of the sheaths of *C. gayana* and *C. ciliaris* than of the sheaths of the temperate grasses may also have contributed to the lower digestibility. The high fibre and lignin contents of the stems of *C. gayana* and *C. ciliaris* were associated with low digestibility and contributed to the low whole crop digestibility. The lower digestibility of chopped than of milled stems of *C. gayana*, *C. ciliaris* and *Z. mays* suggests the presence of tightly packed blocks of sclerenchyma cells restricting the access of rumen microbes to cell walls, as illustrated by Wilson & Mertens (1995).

The whole crop digestibility of *Z. mays* was similar to that of the temperate grasses (Wilman *et al*. 1996*a*). This rather high digestibility was evident in the leaf blades, excluding the midribs, and in the leaf sheaths and stems, when the samples had been milled (Table 3). The proportion of the width of the blades and sheaths occupied by the veins was similar to the proportion in *L. perenne* and *F. arundinacea* and the proportion of the cross-sectional area of the stems occupied by vascular bundles was low (Wilman *et al*. 1996*a*); these factors would contribute to the rather high digestibility of these plant parts and to the moderately low NDF content; the lignin content was also fairly low, particularly in the leaf blades. It seemed from the comparison of milled with chopped forage that rumen microbes can reach the inner cells of the blades and sheaths of *Z. mays* without too much difficulty, even when the pieces of blade and sheath are fairly large, but that there can be considerable difficulty in reaching the inner cells of the stems if the pieces of stem are not broken down to sufficiently small particles; the midribs of the leaf blades may also need to be broken down into small particles if access by microbes is to be satisfactory.

The high digestibility and low NDF content of *Trifolium repens* whole crop samples, noted by Wilman *et al*. (1996*a*), were evident in the individual plant parts, leaflets, petioles and stolons (Tables 3 and 4). It seems to be characteristic of this species that it has a high proportion of cell content in DM (which will be almost completely digestible), combined with a high proportion of lignin in NDF (Table 4 and Wilman & Altimimi 1984) and rather low digestibility of NDF compared with vegetative, temperate grasses (Wilman & Altimimi 1984; Wilman *et al*. 1996*b*). The leaflets of *T. repens* had an appreciably lower NDF content than the stolons and petioles and a rather lower lignin content in DM and yet were, if anything, less digestible than the stolons and petioles; this may suggest some chemical inhibitors of digestion in the leaflets. The proportion of cross-sectional area occupied by vascular bundles was much lower in the stolons of *T. repens* than in stems of *Medicago sativa* of similar diameter (Wilman *et al*. 1996*a*); presumably stolons require less strengthening tissue than upright stems and, at least in the case of *T. repens*, this leads to high digestibility.

The much lower digestibility of stems than leaflets in *M. sativa* would be expected (Terry & Tilley 1964; Wilman & Altimimi 1984), as would the higher NDF and lignin content of stems than leaflets (Wilman & Altimimi 1984). The concentration of lignin in the NDF in the stems of *M. sativa* may have been sufficiently high to prevent digestion of xylem completely, as suggested by Wilson & Mertens (1995). The same may apply in the stems of *D. intortum* and *B. napus*, which were equally high in the concentration of lignin in NDF (Table 4). It should be noted, however, that the crops were grown at higher temperatures than would be recorded in the field in the UK and the higher temperatures would probably have increased the intensity of lignification (Wilson *et al*. 1991). It seemed from the digestibility comparison of milled with chopped stems of the dicotyledonous species that there was nothing like the problem of limited access to cell walls by rumen microbes in chopped material which seemed to be a major reason for the much lower digestibility of chopped than milled stem in *Z. mays*. Wilson & Hatfield (1997) have drawn attention to some of the differences in stem development between legumes and grasses which affect fibre degradation by rumen microflora.

The low digestibility of *D. intortum*, noted by Wilman *et al*. (1996*a*), was evident in all the plant parts examined, leaflets, petioles and stems. Although

there was a considerable difference between the plant parts in the concentrations of NDF and lignin in DM, in the order leaflets < petioles < stems, there was very little difference between plant parts in the *in vitro* digestibility of milled forage. *D. intortum* is a species with a rather high tannin content (Rotar 1965), which is likely to be one reason for its low digestibility (Minson 1990); the concentration of tannin in leaves is likely to be much higher than in stems (Rotar 1965), which may be the reason why the leaflets were no more digestible than the stems when both were dried and milled. The physical structure of *D. intortum* seemed quite favourable from the point of view of digestibility, e.g. the proportion of the cross-sectional area of the stems occupied by vascular bundles was as low as in *T. repens* and the distance between adjacent secondary veins in the leaflets was greater than in *T. repens* (Wilman *et al.* 1996*a*); selection to reduce the chemical inhibition of digestion may be worthwhile.

B. napus was outstanding among the whole crops studied in having high digestibility and low fibre content (Wilman *et al.* 1996*a*). Armstrong *et al.* (1993) recorded high *in vivo* and *in vitro* digestibility of leaf blade, petiole and stem of *B. napus*. In the present study, the high digestibility was evident in all the plant parts examined, particularly in the petioles. Both the petioles and the stems had a particularly low percentage of cross-sectional area occupied by vascular bundles (Wilman *et al.* 1996*a*). The order of the plant parts in terms of the concentrations of NDF and lignin in DM was leaf blades < petioles < stems. As in the case of *D. intortum*, however, the digestibility of milled leaf blades was no higher than that of stems, suggesting some chemical inhibitors of digestion in the leaf blades. Leaf blades with a very low proportion of fibre presumably need some form of protection from predators.

We are grateful to E. J. Mtengeti for his co-operation in making samples available, to N. T. Faithfull and other members of the University of Wales, Aberystwyth, staff for facilities, advice and assistance and to the Ministry of Culture and Higher Education of the Islamic Republic of Iran for financial support.

REFERENCES

Armstrong, R. H., Beattie, M. M. & Robertson, E. (1993). Intake and digestibility of components of forage rape (*Brassica napus*) by sheep. *Grass and Forage Science* **48**, 410–415.

Hacker, J. B. & Minson, D. J. (1981). The digestibility of plant parts. *Herbage Abstracts* **51**, 459–482.

Jones, D. I. H. & Bailey, R. W. (1972). The hydrolysis of cell wall polysaccharides from freeze-dried and oven-dried herbage by rumen and mould carbohydrases. *Journal of the Science of Food and Agriculture* **23**, 609–614.

Jones, D. I. H. & Moseley, G. (1993). Laboratory methods for estimating nutritive quality. In *Sward Measurement Handbook*, *2nd Edn* (Eds A. Davies, R. D. Baker, S. A. Grant & A. S. Laidlaw), pp. 265–283. Reading: British Grassland Society.

Minson, D. J. (1990). *Forage in Ruminant Nutrition*. London: Academic Press.

Rotar, P. P. (1965). Tannins and crude proteins of tick clovers (*Desmodium* spp.). *Tropical Agriculture*, *Trinidad* **42**, 333–337.

Terry, R. A. & Tilley, J. M. A. (1964). The digestibility of the leaves and stems of perennial ryegrass, cocksfoot, timothy, tall fescue, lucerne and sainfoin, as measured by an *in vitro* procedure. *Journal of the British Grassland Society* **19**, 363–372.

Tilley, J. M. A. & Terry, R. A. (1963). A two-stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society* **18**, 104–111.

Van Soest, P. J. (1963). Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *Journal of the Association of Official Agricultural Chemists* **46**, 829–835.

Van Soest, P. J. & Wine, R. H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Journal of the Association of Official Analytical Chemists* **50**, 50–55.

Van Soest, P. J. & Wine, R. H. (1968). Determination of lignin and cellulose in acid-detergent fiber with permanganate. *Journal of the Association of Official Analytical Chemists* **51**, 780–785.

Wilman, D. & Altimimi, M. A. K. (1984). The in-vitro digestibility and chemical composition of plant parts in white clover, red clover and lucerne during primary growth. *Journal of the Science of Food and Agriculture* **35**, 133–138.

Wilman, D., Mtengeti, E. J. & Moseley, G. (1996*a*). Physical structure of twelve forage species in relation to rate of intake by sheep. *Journal of Agricultural Science*, *Cambridge* **126**, 277–285.

Wilman, D., Gao, Y. & Altimimi, M. A. K. (1996*b*). Differences between related grasses, times of year and plant parts in digestibility and chemical composition. *Journal of Agricultural Science*, *Cambridge* **127**, 311–318.

Wilson, J. R. (1976*a*). Variation of leaf characteristics with level of insertion on a grass tiller. I. Development rate, chemical composition and dry matter digestibility. *Australian Journal of Agricultural Research* **27**, 343–354.

Wilson, J. R. (1976*b*). Variation of leaf characteristics with level of insertion on a grass tiller. II. Anatomy. *Australian Journal of Agricultural Research* **27**, 355–364.

Wilson, J. R. & Hatfield, R. D. (1997). Structural and chemical changes of cell wall types during stem development: consequences for fibre degradation by rumen microflora. *Australian Journal of Agricultural Research* **48**, 165–180.

Wilson, J. R. & Mertens, D. R. (1995). Cell wall accessibility and cell structure limitations to microbial digestion of forage. *Crop Science* **35**, 251–259.

Wilson, J. R., Deinum, B. & Engels, F. M. (1991). Temperature effects on anatomy and digestibility of leaf and stem of tropical and temperate forage species. *Netherlands Journal of Agricultural Science* **39**, 31–48.