ELSEVIER

Thermochimica Acta 383 (2002) 161–168

thermochimica acta

www.elsevier.com/locate/tca

Thermogravimetric analysis of perennial ryegrass: relationship between dry matter digestibility and thermal profiles

R.M. Mellon[a,b], H.S.S. Sharma[a,b,*]

[a]*Department of Applied Plant Science, The School of Agriculture and Food Science, The Queens University of Belfast, Newforge Lane, Belfast BT9 5PX, UK*

[b]*Applied Plant Science Division, Department of Agriculture for Northern Ireland, The Queens University of Belfast, Newforge Lane, Belfast BT9 5PX, UK*

Received 4 June 2001; accepted 10 July 2001

Abstract

Derivative thermogravimetric (DTG) analysis was carried out on a set of dried perennial ryegrass (*Lolium perenni*) samples with known dry matter digestibility in the 60, 70 and 80% ranges as determined by acid pepsin and cellulase assays. This analysis was undertaken to determine relationships between DTG weight loss parameters and dry matter digestibility (DMD). The effects of three (5, 20 and 500 °C/min) heating rates under dynamic and isothermal runs were investigated to identify optimum experimental conditions. The weight loss in a minor peak associated with the primary peak, under both dynamic and isothermal conditions was reduced with increasing digestibility of the grass samples and additional effects on both the primary and secondary combustion peaks were also observed. The results of this study suggested that DMD values for grasses could be determined from the thermograms. The advantages of using different heating rates with associated effects on the thermal profile of the different fibre fractions are discussed. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Derivative thermogravimetric analysis; Perennial ryegrass; Dry matter digestibility

1. Introduction

Digestibility of grass is expressed as a percentage of dry matter digestibility (DMD) These values are important to farmers, ruminant nutritionists, agronomists and grass breeders. DMD is an indicator of animal production potential and as such is an important factor for the selection of grass varieties for breeding. DMD is primarily determined by lengthy wet chemistry assays, which are expensive in terms of man-hours, equipment and materials required. The main components of dried perennial ryegrass are cell wall contents and cell walls comprised primarily of holocellulose, (hemicellulose/cellulose) and lignin [1]. Lignin sets the limits of digestibility but has no effect on the availability of carbohydrate within or outside the cell wall and whether lignin has a uniform effect upon biodegradation is uncertain [2]. The in vitro digestibility values assigned to grasses using acid pepsin and cellulase digestion techniques [3] are influenced by several factors. Significant among these is the degree of lignification which takes place on maturation, as the proportion of the cell wall fraction relative to the cellular contents increases resulting

* Corresponding author. Tel.: +44-2890-255247; fax: +44-2890-255007.
E-mail address: s.sharma@qub.ac.uk (H.S.S. Sharma).

0040-6031/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0040-6031(01)00681-5

in a reduction of digestibility [4]. Lignin is often referred to as non-core lignin consisting of ferulic and *p*-coumaric acids and core lignin made up of phenylpropanoid polymer [5]. With maturation, the holocelluloses become more structural as ferulic acid ester and ether bonds to arabinoxylans and become covalently cross-linked to lignin monomers [6] resulting in secondary wall thickening. Maturation leads to an altering of the relative ratios of ferulic and coumaric acids [7] present, a decrease in the ester linked ferulic acid readily accessible by hydrolysis and an increase in the insoluble lignin content resulting in a reducing of DMD values [5,7].

These factors could ultimately change the thermal properties of the fibre fractions in grass. The use of derivative thermogravimetry (DTG) for assessment of microbial breakdown of mushroom compost [8], grading of flax fibre quality [9] and changes in fibre fractions in wheat straw as a result of fungicide and growth hormone treatments [10] has been previously reported. The relationship between degree of digestibility and rate of combustion of the major components of grass has not been previously investigated. The effect of using different heating rates together with dynamic and isothermal techniques were carried out in order to determine optimal conditions for examining differences in the fibre fractions present in perennial ryegrass and their relationship with digestibility.

2. Materials and methods

2.1. Assessment of dry matter digestibility

Samples (50) of perennial ryegrass (*Lolium perenni*) of differing varieties and cuts were obtained from grass trial plots at the North Ireland Horticulture and Plant Breeding Station at Loughgall. The samples were dried at 105 °C and milled using a cyclotec mill (sieve 0.5 mm) prior to analysis to determine their DMD values using an in vitro 24 h acid pepsin/24 h cellulase digestion technique [3]. An amount of 25 ml of acidified pepsin solution (Sigma P-7125) 1:25,000 from porcine stomach mucosa) were added per 0.2 g of dried milled grass sample, and the samples were placed in an incubator at 48 °C for 24 h, each sample run in triplicate. After this time, the samples were corrected to pH 4.7 through addition of a quantity of 1 M calcium carbonate solution determined by adjustment of blank incubated acidified pepsin solutions. Cellulase (Sigma C-9422) from *Trichoderma viride* working solution was then added, 38.5 ml per sample and the samples returned to the incubator for a further incubation of 24 h. Undigested material was recovered by suction filtration through scintered glass crucibles, washing with distilled water drying at 105 °C

$$\text{pepsin/cellulase DMD (\%)} = \left(\frac{(\text{crucible} + \text{residue weight}) - \text{crucible weight}}{\text{sample weight}}\right) \times 100$$

Nine samples were selected, three samples in the 60%, three in the 70% and three in the 80% digestibility ranges for further analysis by derivative thermogravimetry.

2.2. Thermogravimetric analysis of grass

The milled samples were further ground for 5 min with a mortar and pestle to ensure consistency in sample preparation. The samples (3–3.2 mg) were suspended into a furnace in an alumina crucible from a Mettler (TG 50 MT5) microbalance using a computer-controlled temperature processor. A range of heating rates and isotherms were selected [9]. The dynamic runs were at heating rates of 5, 20 and 50 °C/min, through a temperature range of 32–600 °C at an airflow rate of 20 mm/min. Isothermal runs were also carried out at 5, 20 and 50 °C/min heating rates, under the same temperature range. The isothermal differed from the dynamic analytical runs in requiring a temperature hold at 230 °C for 10 min, before returning to the previous heating rate. The TG curves were assessed using Star software (Mettler, Toledo). Analysis for variance was performed on the results and correlation analysis was also carried out to determine relationship between the test parameters.

2.3. Reference samples

The analysis of reference samples under dynamic conditions by DTG enabled interpretation of thermograms and the following materials were tested: alpha

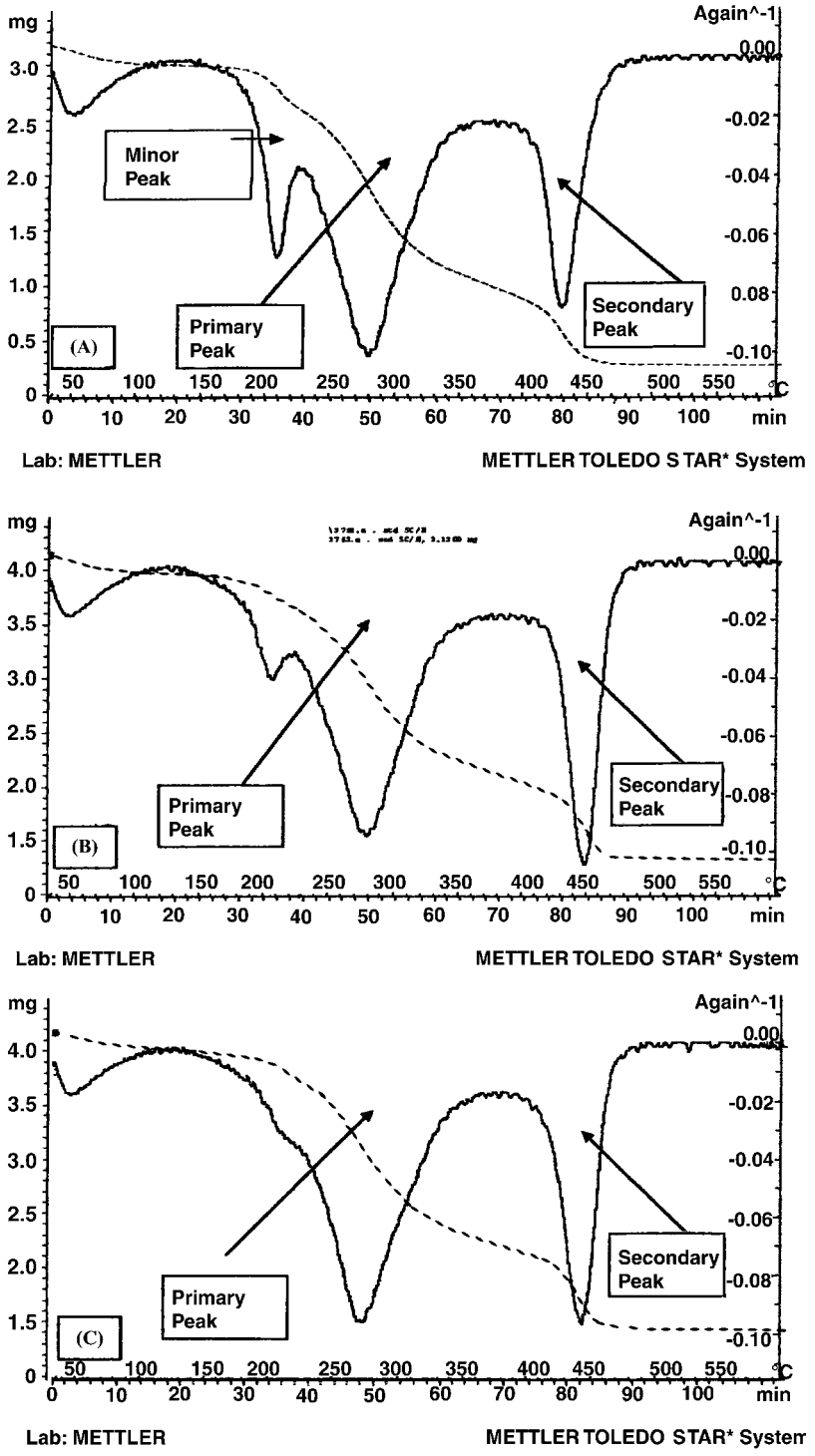

Fig. 1. Dynamic thermograms showing weight loss (dashed line) and derivative curves (solid line) of three perennial ryegrass samples with varying DMD values (A) 66%; (B) 79%; and (C) 83% as determined by pepsin–cellulase assay.

cellulose (Sigma C-8002), L-glucosan (A-8417), xylan (Sigma X-0627), araban (Koch-Light 93484), lignosulpfonic acid (Aldrich-Chem, 37097.5), arabinogalactan (Sigma A-9788), D-glucuronic acid (Sigma G-2125), fructose (Sigma F-0127), ferulic acid (Sigma F-3500) and *p*-coumaric acid (Sigma C-9008).

3. Results

3.1. Dynamic analysis

During DTG analysis, the temperature gradually increased and the moisture present in the sample was eliminated at near 90–110 °C. With further increases in temperature from 180 to 400 °C, major combustion of the sample occurred at near 280 °C preceded by a minor peak at 210 °C. The secondary peak extending from 400 to 480 °C showed a peak decomposition temperature at near 430 °C, which was lower in height compared to the primary peak. The profile of low DMD grass was distinctly different from high DMD grass as shown by the gradual reduction of the minor peak to a shoulder associated with the primary peak (Fig. 1a–c). In addition, the secondary peak decomposition temperature shifted from 429 °C for low DMD to 450 °C for medium followed by a reduction to 440 °C for grass of high DMD value. (Fig. 1a–c).

Although the combustion of any individual reference compound gives rise to unique thermal profile, analysis of more complex structures leads to an alteration of properties of its individual components giving rise to a composite thermal profile, within which there is an over-lapping of information. Combustion of carbohydrates, polysaccharides and phenolic acids (ferulic and *p*-coumaric acid) takes place in the temperature band of 180–280 °C. Closer to 280 °C are the more complex polysaccharides, such as amorphous or non-structural hemicellulose. The cellulose, lignin and structural hemicellulose complex predominantly combust in 280–400 and 400–500 °C temperature bands, respectively (Table 1).

The primary peak showing weight loss (WL1a) and height (PH1) was significantly ($P < 0.01$) reduced in low DMD samples but effect on decomposition temperature (PT1) was not apparent (Table 2). The effects of heating rate on the above parameters were variable showing maximum impact on peak height (PH1). Although the effects of digestibility of the samples on WL1b were not significant, the differences caused by heating rate on WL1b were significant ($P < 0.001$). Weight loss (WL2) and peak height (PH2) of the secondary peak were significantly higher in more digestible materials and maximum values of the parameters were observed when analysed at 50 °C/min. The DTG profiles of the dynamic runs at heating rates of 50 and 20 °C/min analyses were similar to the 5 °C/min. The effects of the heating rates on peak decomposition temperature (PT2) were not significantly different, although PT2 for medium DMD samples were significantly higher ($P < 0.001$) than low DMD samples. The residue left in the crucible mainly consisting of inorganic fraction was signifi-

Table 1
Thermogravimetric analysis of reference samples showing initial, maximum and final pyrolysis temperatures and volatilisation of the fractions

	Temperature of active combustion peak (°C)			Volatilisation (%)								
	Initial	Maximum	Final	150	200	250	300	350	400	450	500	550
a-Cellulose	250	346	400	1.6	2.4	3.2	10.0	68.0	73.5	82.9	95.2	95.2
Araban	185	288	400	8.5	9.3	21.8	51.1	65.2	77.9	87.3	96.2	96.2
Arabano galactan	240	315	405	4.2	5.0	7.1	42.1	78.9	86.0	82.9	97.1	97.5
p-Coumaric acid	195	233	330	0.0	0.6	54.5	64.3	67.8	71.0	75.2	94.3	99.3
Ferulic acid	180	240	295	0.0	7.1	89.0	94.5	95.5	98.0	99.0	99.3	99.3
Fructose	160	211	400	0.6	10.6	31.9	56.2	65.6	73.0	80.0	96.3	97.0
D-Glucuronic acid	145	165	190	1.3	39.4	47.4	53.9	61.5	63.5	65.4	66.7	69.2
Inulin	170	245	390	0.0	0.0	20.0	48.0	59.0	65.0	72.0	84.0	86.0
Xylan	250	285	300	9.6	10.2	12.1	64.3	72.2	81.9	87.3	90.5	91.4

Table 2
Effects of dynamic heating rate (5, 20, 50 °C) on DTG parameters of grasses with low (60%), medium (70%), and high (80%) DMD values as determined by pepsin–cellulase assay[a]

DMD	Heating rate (°C/min)	Primary decomposition peak (180–400 °C)				Secondary decomposition peak (400–550°)		
		WL1a (minor peak) (%)	WL1b (main peak) (%)	PH1 (mg/min)	PT1 (°C)	WL2 (%)	PH2 (mg/min)	PT2 (°C)
Low	5	14.87	51.55	0.09	288.7	18.29	0.07	438.1
Low	20	13.38	52.93	0.36	303.1	23.34	0.53	448.2
Low	50	15.10	37.79	0.70	295.7	26.56	0.60	445.9
Medium	5	13.91	48.13	0.09	292.2	20.47	0.09	450.8
Medium	20	11.80	50.28	0.34	306.3	25.08	0.43	472.2
Medium	50	13.78	43.4	0.67	300.1	28.52	0.61	468.3
High	5	12.50	45.97	0.08	282.3	22.55	0.09	452.4
High	20	10.82	45.53	0.31	292.1	29.20	0.36	450.3
High	50	12.91	40.02	0.62	285.1	30.92	0.55	452.9
S.E.M.	Sample	0.534**	1.396 ns	0.008**	0.972***	0.398***	0.018*	2.895***
S.E.M.	Heating rate	0.924*	2.418***	0.014***	1.684***	0.690***	0.030***	5.015 ns

[a] WL1a, weight loss in the minor peak; WL1b, weight loss in the primary peak (PP); PH1, peak height of PP; PT1, peak temperature of PP; WL2 weight loss in the secondary peak (SP); PH2, peak height of SP; PT2, peak temperature of SP; S.E.M., standard error of means; ns, not significant.

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

cantly ($P < 0.05$) higher for high DMD grass materials (Table 2).

3.2. Isothermal analysis

Under isothermal conditions, the Primary peak can be divided into a minor peak extending from 180 to 230 °C and the main peak extending from 230 to 380 °C, and was followed by secondary peak ranging from 380 to 480 °C (Fig. 2a–c). A shoulder at 230 °C for low DMD grass samples followed the peak decomposition temperature for the minor peak at 200 °C. However, the profile of minor peak and shoulder changed in more digestible grass samples, peaking at 230 °C (Fig. 2a–c). The changes in decomposition temperature and general profile of the primary and secondary peaks were similar to results obtained under dynamic conditions.

The weight loss (WL1a) in the minor peak was significantly ($P < 0.05$) higher when analysed at 20 °C/min compared to the other two heating rates for low or high DMD grass samples (Table 3). In contrast, weight loss (WL1b) for the primary peak was significantly ($P < 0.001$) higher when analysed at 5 °C compared to the other two heating rates. Of the three heating rates tested, 50 °C showed significantly ($P < 0.001$) higher peak height (PH1) and decomposition temperature (PT1) for the primary peak, and weigh loss (WL2) and height (PH2) for the secondary peak. Differences in the decomposition temperatures of the secondary peak were not significant.

Under dynamic conditions, DMD of the grass samples correlated with WL1a ($r = -0.605$), WL1b (−0.916) and WL2 (0.939) at a heating rate of 5 °C/min. At 20 °C/min, DMD correlated highly with PH1 (0.836), WL1a (0.602), WL1b (−0.882), WL2 (0.897) and PH2 (−0.838). At 50 °C/min DMD values correlated with WL1a (−0.613), WL2 (0.888) and residue (0.793). When analysed under isothermal conditions at a heating rate of 5 °C, high correlation between DMD and weight losses in both primary (−0.917) and secondary peak (0.898) were observed. At 20 °C/min heating rate, the DMD of the grass samples correlated with WL1b (−0.898), and WL2 (0.899) and to a lesser extent with WL1a (−0.543). Similarly, at 50 °C heating rate, WL1a (−0.700), WL1b (−0.929), WL2 (0.816) and residue (0.827) correlated strongly with DMD values of the grass samples.

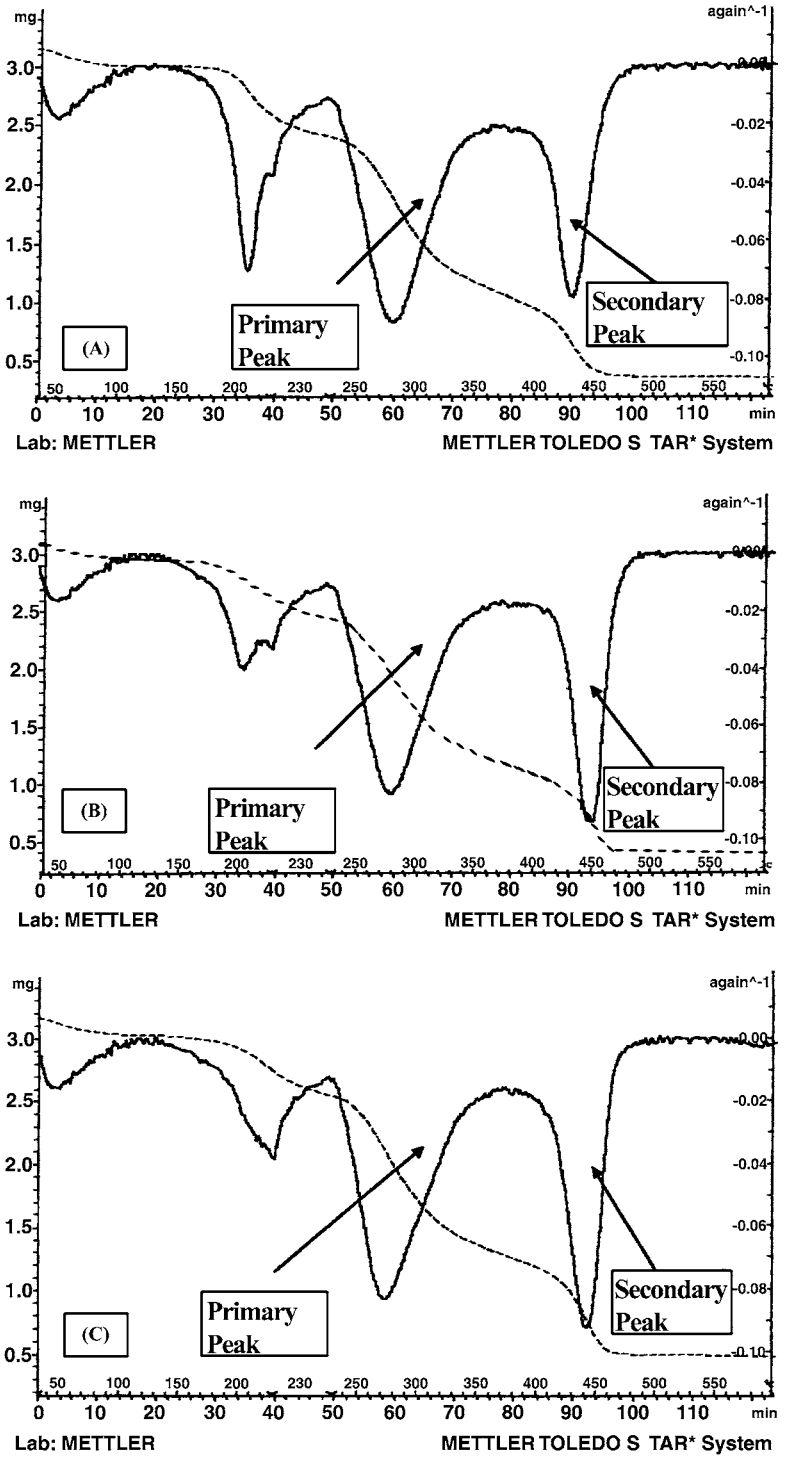

Fig. 2. Isothermal thermograms showing weight loss (dashed line) and derivative curves (solid line) of three perennial ryegrass samples with varying DMD values (A) 66%; (B) 79%; and (C) 83% as determined by pepsin–cellulase assay.

Table 3
Effects of isothermal heating (temperature hold at 230 °C for 10 min) and rates of heating (5, 20, 50 °C/min) on DTG parameters of grasses with low (60%), medium (70%), and high (80%) DMD values as determined by pepsin–cellulase assay[a]

DMD	Heating rate (°C/min)	Primary decomposition peak (180–400°)				Secondary decomposition peak (400–550°)		
		WL1a (Minor peak) (%)	WL1b (Main peak) (%)	PH1 (mg/min)	PT1 (°C)	WL2 (%)	PH2 (mg/min)	PT2 (°C)
Low	5	12.8	54.1	0.09	291.0	18.9	0.07	437.6
Low	20	15.2	43.8	0.34	300.3	27.2	0.43	448.2
Low	50	14.1	42.9	1.12	302.6	28.5	0.63	374.8
Medium	5	11.8	50.4	0.09	291.7	21.0	0.10	450.0
Medium	20	14.0	42.3	0.33	303.5	28.3	0.40	465.6
Medium	50	12.8	41.5	1.06	305.5	30.0	0.64	460.3
High	5	10.5	48.7	0.07	283.7	23.0	0.09	449.9
High	20	13.2	40.5	0.33	294.0	30.2	0.36	461.8
High	50	11.2	38.7	1.04	298.5	31.8	0.56	450.0
S.E.M.	Sample	0.515*	0.408***	0.013***	0.899***	0.516***	0.015 ns	13.07 ns
S.E.M.	Heating rate	0.893*	0.707***	0.022***	1.556***	0.893***	0.025***	22.64 ns

[a] WL1a, weight loss in the minor peak; WL1b, weight loss in the primary peak (PP); PH1, peak height of PP; PT1, peak temperature of PP; WL2 weight loss in the secondary peak (SP); PH2, peak height of SP; PT2, peak temperature of SP; S.E.M., standard error of means; ns, not significant.

* Significant at $P < 0.05$.

*** Significant at $P < 0.001$.

4. Discussion

Within the temperature band of 180–400 °C, carbohydrates, non-structural hemicelluloses and cellulose burn in an order of increasing thermal stability. The less stable, weak bonded complexes, combust at the lower temperatures. As cross-linking of fibre fractions increases through intra-molecular bonding, these complexes, structural hemicelluloses and lignin, become more thermally stable and higher temperatures (400–600 °C) are required for their combustion [8,11].

Both lignin and hemicellulose have complex structures, hemicellulose being the most intricate of the plant polysaccharides and lignin, a condensed phenylpropanoid polymer thought of more as a class of substances rather than a single substance [2]. The composition of both hemicellulose and lignin, and their negative effect on digestibility was reported to vary with grass maturation [1]. A decrease in grass digestibility with maturation has been linked to increasing lignification together with the development of ferulic acid cross-links [12]. It appears that with maturation, secondary cell wall thickening occurs, as hemicellulose and lignin complexes become increasingly cross-linked, rendering them less vulnerable to enzymatic degradation.

The minor peak (210 °C) associated with the primary peak under both dynamic and isothermal conditions diminished with increasing digestibility and might be related to the availability of total degradable cell wall materials. The fractions oxidising in this peak are probably carbohydrates, such as fructose and low molecular weight phenolic acids. The carbohydrates were oxidised at the onset of combustion, peaking near 210 °C and followed by the phenolic acids as shown by well-defined peaks at 233 and 239 °C for the *p*-coumaric and ferulic acid reference standards, respectively. The phenolic acids present in the test samples have been quantified using capillary electrophoresis and results have revealed that concentrations of both acids were reduced in samples with high DMD values compared to poorly digestible materials (unpublished data). The changes in the thermal profiles and associated reduction in weight losses in the minor peak and the primary peak could be linked to lower phenolic acid content in highly digestible grass.

The relationship between weight loss in the secondary peak and DMD values of the samples was found to be significant. If the fractions related only to indigestible lignin, their relationship to DMD should be inversely related rather than the proportional

increase observed in high DMD grass. This would suggest that weight loss in the secondary peak might not only include lignin and hemicelluloses but also other high molecular weight fractions of unknown structure. The observed increase in inorganic fraction in more digestible materials suggest that certain divalent cations, such as Ca, K and Na may play a significant role in the availability of the substrate.

The use of isothermal methods has enabled clearer separation of the components of the minor peak at 210 °C and the associated shoulder at 230 °C became more prominent as digestibility increases. The changes in the thermal profiles of the test samples are consistent with differences in the DMD values, but their interpretation is difficult. This technique, when further investigated and supported by wet chemical analysis will enable a clearer understanding of compositional differences and thermal properties.

The observed relationship between weight losses and DMD values of the samples suggest that digestibility of grasses could be predicted from the thermogravimetric data, though a much larger sample set would be necessary for the statistical integrity required of any such model. Although a heating rate of 5 °C/min was the best in terms of amplifying differences in the fibre fractions, a medium rate of 20 °C/min would be better due to the inevitable constraints on time. Further work on both wet chemistry and instrumental analysis including DTG–MS and mid-infrared microspectroscopy assessment [13] would enable a better interpretation of the thermal profiles and a clearer understanding of their relationship to digestibility.

Acknowledgements

We wish to thank Dr. G. Lyons for assistance in the analysis of samples and D. McCall for helpful comments.

References

[1] W. Holmes, Grass. Its Production and Utilisation, Blackwell Science, 1980.
[2] P.J. Van Soest, Nutritional Ecology of the Ruminant, O&B Books Inc., Corvallis, Oreagon, 1992.
[3] D.I.H. Jones, M.V. Hayward, J. Sci. Food Agric. 24 (1973) 1419–1426.
[4] K.W. Moir, J.R. Wilson, G. W Blight, J. Agric. Sci. Camb. 88 (1977) 217–222.
[5] D.R. Buxton, L.R. Russell, Crop. Sci. 28 (1988) 553–558.
[6] R.D. Hatfield, J. Ralph, J. Sci. Food Agric. 79 (1999) 403–407.
[7] R.D. Hartley, J. Sci. Food Agric. 23 (1972) 1347–1354.
[8] H.S.S. Sharma, Thermochim. Acta 285 (1996) 211–220.
[9] H.S.S. Sharma, G. Faughey, D. McCall, J. Textile Ins. 87 (1996) 249–257.
[10] H.S.S. Sharma, Ann. Appl. Biol. 137 (2000) 297–303.
[11] H.S.S. Shaima, Mushroom Sci. 14 (1995) 267–273.
[12] T. Kondo, K. Mizuno, T. Kato, Can. J. Plant Sci. 71 (1990) 495–499.
[13] D.E. Akin, D.S. Himmelsbach, R.T. Carl, W.W. Hanna, F.E. Barton II, Agro. J. 85 (1993) 171–175.