Digestion kinetics of mature grasses

A.V. CHAVES[1,2], G.C. WAGHORN[1], I.M. BROOKES[2] AND J.L. BURKE[1,2]

[1]AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand[2]

ABSTRACT

Digestion kinetics were measured for mature (green and non-senescent) components of five grass species using *in sacco* and *in vitro* incubations to define rates of degradation and nutrient release. The data will be incorporated into a dairy nutrition simulation model to identify limitations in nutrient supply to cows grazing mature pasture in late spring. Perennial ryegrass, tall fescue, Yorkshire fog, phalaris and paspalum were hand separated into leaf, stem and inflorescence for incubations. Percentages of fibre (NDF) in DM fractions ranged from 50-69% (leaf), 63-75% (stem) and 50-68% (inflorescence). Crude protein concentrations in the DM of the respective fractions were 7.5-23.7%, 3.8-8.3% and 7.9-12.3%. Soluble DM (% of the total) determined after mincing accounted for 31-53% of leaf, 26-55% of stem and 20-48% of inflorescence, and fractional (h^{-1}) degradation of the insoluble DM was very slow, ranging from 0.034-0.113 (leaf), 0.025-0.036 (stem) and 0.033-0.072 (inflorescence). After 24 hours of *in vitro* incubation plant nitrogen content become limiting for fermentation in most instances.

Keywords: forages; digestion kinetics; *in sacco*; *in vitro;* plant maturity; dairy cows.

INTRODUCTION

A major issue facing the New Zealand dairy industry is the rapid decline in milk production after peak lactation and anoestrous corresponding with grass maturation in October-December. Grasses commence spring growth with vigorous leaf production, which has a high nutritive value (feeding value x voluntary intake) for grazing sheep and cattle, but as daily temperature rise an increasing proportion of stem and inflorescence appears. Although the extent to which grass is allowed to produce seed heads can be controlled by grazing management, nutritive value declines because of changes in chemical composition. The principal changes are increased proportions of fibrous stem (Wilman & Agiegba, 1982), and decreased concentrations of leaf protein, so that the maturing plant has higher proportions of fibre and lower proportions of soluble (readily fermentable) carbohydrate and protein in the dry matter. These changes reduce the amount of amino acids available to ruminants and may increase the proportions of acetate:propionate available for absorption (Russell & Strobel, 1993). However, the most significant effect of grass maturation is that the rate of digestion and clearance of residual forage fibre from the rumen is reduced, because mature forages are slower to digest and require more chewing to reduce the particle size of plant fragments to a size able to pass out of the rumen (Ulyatt *et al*., 1986). The slower rate of passage from the rumen reduces feed intake. Hence, maturation in a grass-dominant sward results in lowered intake as well as declining nutritive value.

Grass maturation has a major impact upon dairy cow productivity because grass-dominant pasture is unable to provide sufficient nutrients to match the genetic merit of New Zealand dairy cows, as evidenced by low milk production compared to cows fed concentrate diets and anoestrus coinciding with pasture maturation in some situations (Verkerk *et al*., 2000).

Improved nutrition for dairy cows requires an understanding of digestion kinetics, including the rate of degradation of plant constituents and the nutrients released from digestion. This is especially important with grass dominant pastures where composition changes with the time. Burke *et al*. (2000) defined the degradation kinetics of immature leafy material from a range of grasses, legumes and herbs to be used as a basis for formulating forage based total mixed rations (TMR). That work determined degradation rates for dry matter, protein, soluble carbohydrate, neutral detergent fibre (NDF; cellulose, hemicellulose and lignin) and acid detergent fibre (ADF; cellulose and lignin). That study also indicated the amount and proportions of volatile fatty acids (VFA) produced, and has provided a mathematical basis for comparing contrasting feed types, but only for immature leaves. The work described here examines grasses which have not been grazed and are in an advanced stage of maturity, not senescent but with stems and nearly mature flowers. This study aims to determine the digestive characteristics at the opposite end of the range to that of Burke *et al*. (2000), using five very mature grass species.

This study involved the separation of the five grass species into leaf, inflorescence and stem (including sheath) fractions for incubation *in vitro* and *in sacco*. *In vitro* incubations were conducted to determine the products of degradation (ammonia from proteolysis and VFA), whilst the *in sacco* technique was conducted to determine the rate at which dry matter (DM) and its constituent chemical fractions are degraded through microbial digestion.

METHODS

The mature grass species used in this study were perennial ryegrass (*Lolium perenne* L. cv. Grasslands Samson), tall fescue (*Fescue arundinacea*), Yorkshire fog (*Holcus lanatus*), phalaris (*Phalaris aquatica*) and paspalum (*Paspalum dilatatum*). About 2 kg of each species was harvested in the summer of 1999/2000, refrigerated and immediately separated (by hand) into leaf, inflorescence and stem (with sheath) fractions, and stored at -16 ^{o}C until incubation. Dead matter was discarded. Approximately 500g wet material was obtained for each plant fraction for *in sacco* and *in vitro* incubations of minced material as well as measurements of dry matter content, particle size

[2] Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.

distribution of minced fractions and chemical composition by near infra red reflectance spectroscopy (NIRS). These procedures have been described by Burke *et al.* (2000) for digestion of immature forages.

Frozen forages were chopped into approximately 2 cm lengths (scissors) and minced (whilst frozen) in a Kreft Compact meat mincer with 12 mm holes in the sieve plate. Minced material was stored at -16 ^{o}C until the day prior to incubations when about 2.5g wet weight (ww; 0.5g DM) was placed in incubation bottles and 25g ww (5.0g DM) into 100 x 100mm dacron bags for placement in the rumen of a fistulated cow. *In sacco* and *in vitro* incubations were carried out simultaneously of leaf, stem and inflorescence fractions of one species during each incubation. One ruminally cannulated non-lactating Friesian cow was fed lucerne hay for all incubations in order to maintain a similar rumen environment over the period of evaluation and inclusion of ryegrass standards enabled adjustment for between run variations.

Fourteen bags of each forage constituent were placed in the rumen and duplicates removed after 0, 2, 6, 12, 24, 48 and 72h for washing, drying (60°C), weighing and analysis of residues by NIRS. Disappearance of DM and other fractions were analysed using a non-linear model described by Burke *et al.* (2000) to determine fractional disappearance rate (k, %/hour), lag time (L, hour) and potential degradation (P) according to:

$$P = A + B\ (1\text{-}e^{-k(t-L)})$$

where A = soluble DM (% of DM at t=0h), B = degradable insoluble DM and t is time in hours.

Twenty-one bottles were prepared with each forage constituent for *in vitro* incubations by adding 12ml buffer, 0.5ml reducing agent and 3ml rumen fluid to the plant material to 50ml bottles (Burke *et al*., 2000). Bottles were made anaerobic by flushing with carbon dioxide and held at 39∞C in an oscillating incubator for the duration of each incubation. Triplicate samples were removed after 0, 2, 6, 8, 12, 24, 36 and 48h of incubation for determination of ammonia concentrations and VFA (Burke *et al*, 2000) at 0, 6, 12, 24 and 48h.

RESULTS

Although dead matter was discarded during the hand separation of leaf, stem and flower, the high DM percentage of most components, together with NDF concentrations in excess of 50% of the DM, demonstrate the maturity of the forages collected (Table 1). Paspalum had the highest NDF concentrations (over 66% of the DM for all constituents), with a low crude protein (CP) concentration (below 8% of the DM). Leaf of temperate forages (ryegrass, tall fescue, Yorkshire fog and phalaris) had 15% or more CP, with lower concentrations in flowers and low (<10%) and variable CP concentrations in stem. Soluble carbohydrate concentration was low (<10%) in all plant components, except ryegrass and tall fescue flowers (Table 1). Stem accounted for 40-60% of plant DM across all grasses but leaf ranged from 8-40% and flower 12-30% of the DM. Mature ryegrass and Yorkshire fog had over 35% leaf when mature, whereas fescue and phalaris had less than 15% leaf and over 30% flower dry matter.

The particle size distribution of minced material (% of DM) showed that 18-25% of leaf and stem fractions of the temperate forages were retained on sieves with 2 mm or large aperture sizes, but a higher proportion of flower (20-38% of DM) was retained on these sieves. In contrast only 6-12% of paspalum fractions were 2 mm or larger in size. Dry matter passing sieves with a 0.25 mm aperture size (fine particulate and soluble DM) accounted for 25-55% of plant DM, with a narrow range across the five grass species for flowers (35-47%) relative to leaves (tall fescue 25% - Yorkshire fog 53%) and stems (tall fescue 29% - Yorkshire fog 55%). Intermediate sized DM (0.25 – 2.0 mm) accounted for 25-53% of DM across leaf, stem and flower fractions of the five grasses.

TABLE 1: Dry matter percentage (DM%) at harvest, chemical composition (g/100g DM) and predicted organic matter digestibility (g/100g) of leaf, flower and stem fractions of five mature grasses used for measurement of digestion kinetics.

	DM (%)	CP	CHO	NDF	ADF	OMD
Perennial ryegrass						
leaf	21.9	18.9	8.7	49.2	23.7	79
flower	45.5	12.3	18.1	52.7	52.7	63
stem	30.2	8.3	6.3	63.5	38.1	56
Tall fescue						
leaf	30.5	15.2	4.9	57.6	34.0	63
flower	46.7	9.8	18.1	50.6	33.6	60
stem	36.1	7.9	6.3	63.6	38.2	57
Yorkshire fog						
leaf	20.3	23.7	6.2	50.0	24.3	78
flower	54.3	8.8	5.9	60.0	34.8	57
stem	34.1	5.6	4.7	68.2	42.3	5.3
Phalaris						
leaf	32.2	21.7	5.9	51.0	31.7	7.2
flower	37.6	10.4	9.2	49.9	33.3	60
stem	34.6	7.1	0.0	72.5	44.5	46
Paspalum						
leaf	39.3	7.5	0.0	69.0	42.4	53
flower	50.7	7.9	0.0	68.5	44.3	39
stem	28.6	3.8	6.5	66.9	41.8	55

Abbreviations: CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; CHO, soluble carbohydrates; OMD, organic matter digestibility

TABLE 2. Mature grass dry matter (DM) degradation characteristics (% of DM) defined as soluble DM (A), degradable insoluble DM (B), potential degradability (P), fractional degradation rate (K, h^{-1}), lag time (L, h) and effective degradability (E) which takes into account the effect of passage rate from the rumen.

	A	B	P	K	L	E[1]
Perennial ryegrass						
leaf	39	50	89	0.105	4.4	68
flower	48	35	83	0.037	4.1	59
stem	47	35	82	0.025	6.0	54
Tall fescue						
leaf	31	47	78	0.085	4.4	54
flower	27	47	74	0.072	0.0	5.3
stem	26	36	62	0.036	5.5	36
Yorkshire fog						
leaf	38	51	89	0.113	3.9	68
flower	45	28	73	0.053	4.0	56
stem	35	45	80	0.031	5.9	46
Phalaris						
leaf	43	51	94	0.034	0.1	63
flower	31	55	86	0.033	0.2	51
stem	35	45	80	0.031	5.9	46
Paspalum						
leaf	53	39	92	0.046	6.0	66
flower	20	42	62	0.044	4.7	35
stem	55	36	91	0.033	4.7	65

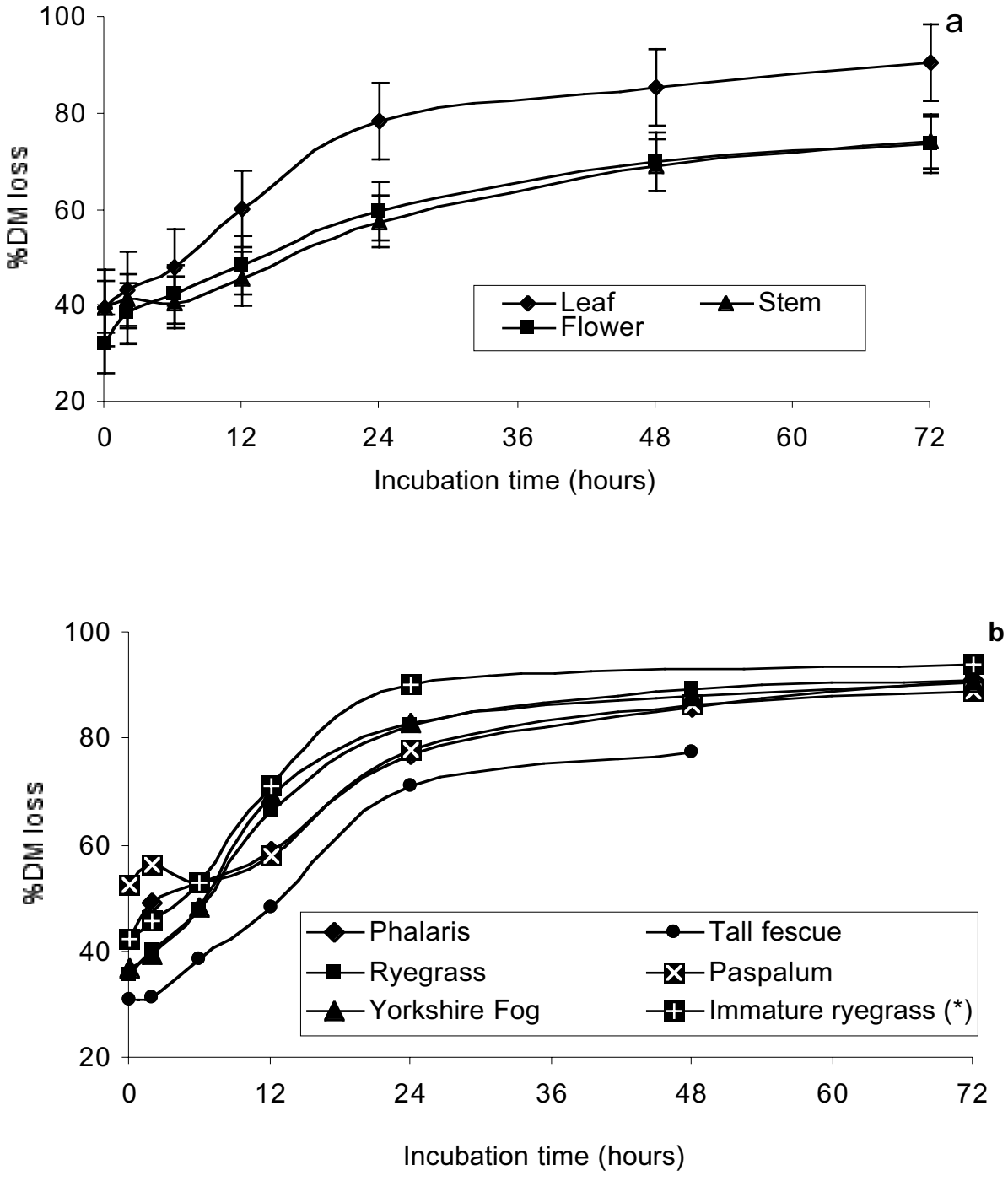
[1]Calculated using a fractional passage rate of 0.06h^{-1}

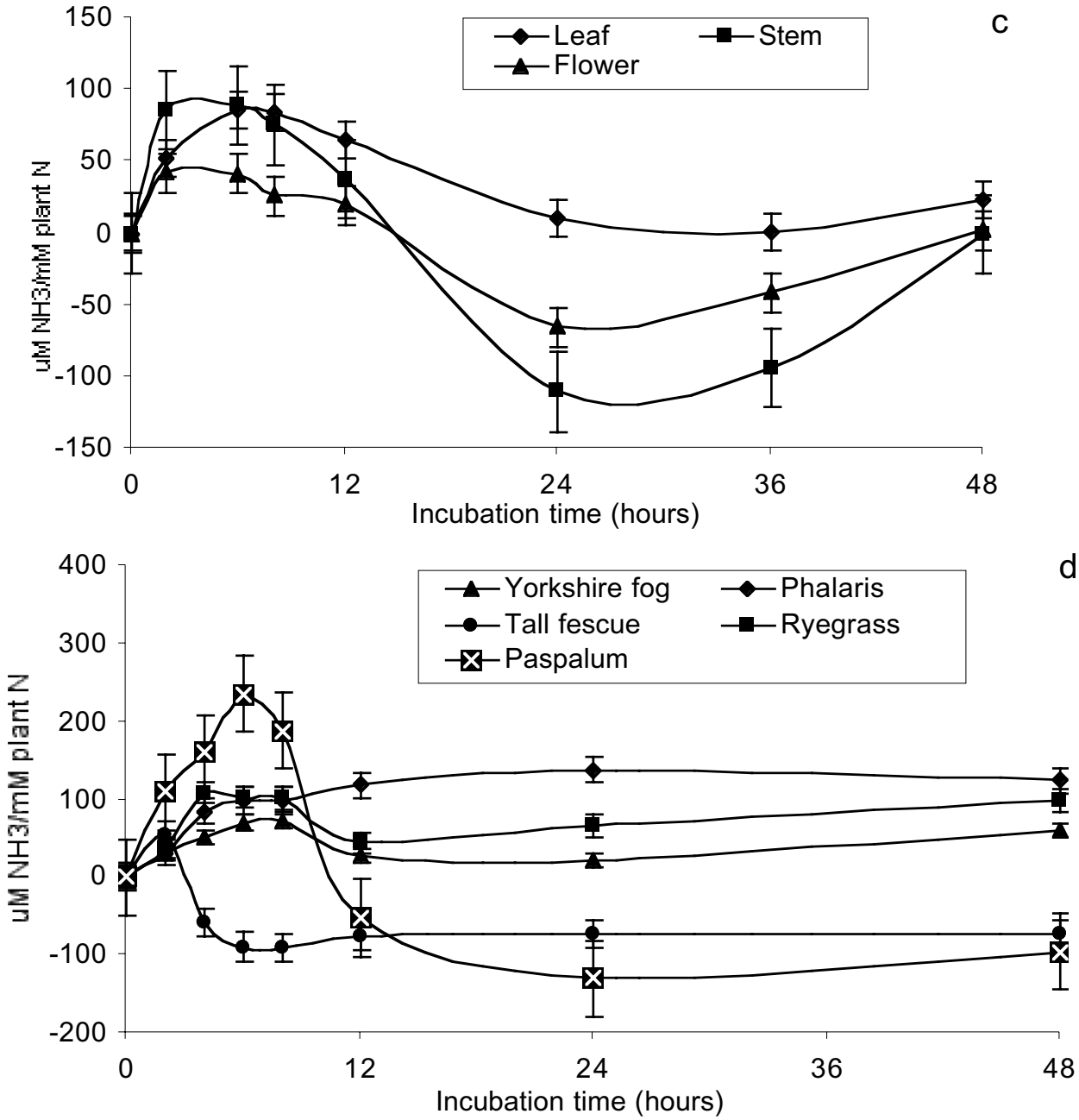
In sacco dry matter disappearance data are summarised in Table 2 and illustrated in Figure 1. Figure 1a shows slow but similar rates of degradation for both flower and stem fractions average for the five forages, relative to leaf. The error bars indicate substantial differences between grass species in degradation rate of individual constituents, and this is further demonstrated by the DM disappearance from the leaf fraction of each species in Figure 1b.

Kinetic data based on fitting curves to *in sacco* data (Table 2) show rapid degradation rates of ryegrass, fescue and fog leaf and very slow degradation of phalaris and paspalum leaf. Leaf, stem and flowers had similar, slow degradation rates for phalaris and paspalum and differences in effective degradability (a prediction of degradation *in vivo*) were due in part to particle size reduction and release of soluble DM during mincing or chewing. Paspalum flower was predicted to be especially indigestible (E=35%) *in vivo* compared to flower from temperate grasses (E=51-59%). Most constituents had substantial lag periods prior to DM loss, suggesting a relatively slow colonisation of particles by rumen bacterial and fungi.

Products of degradation are indicated (Figure 1c) by net ammonia production from plant components averaged across species and from leaves of each species (Figure 1d).

FIGURE 1: *In sacco* dry matter disappearance from (a) leaf, stem and flowers from five mature grasses (mean ± SD), (b) leaf from each grass species and *in vitro* ammonia production from (c) leaf, stem and flowers from five mature grasses (mean ± SD), and (d) leaf from each grass species. (* - Burke *et al*., 2000)





Net production excludes plant nitrogen incorporated into bacterial protein (Barrell *et al*., 2000) but the net ammonia production from mature leaves (Figure 1c) suggests N content would not limit bacterial growth. This contrasts with the negative ammonia values for stem and flower after 12h of incubation when N supply was insufficient for microbial growth (negatives values indicate ammonia uptake from the rumen innoculum). Because of the N induced limitations associated with stem and flower comparative data for the five grass species have only been given for leaf fractions (Figure 1d) and these data show insufficient N was released from both tall fescue and paspalum to sustain microbial growth. Nitrogen insufficiency will limit degradation rates *in situ*.

TABLE 3. Volatile fatty acid (VFA) production from *in vitro* incubations after 12 hours (mmol/g dry matter) and ratios of acetate:propionate (A:P) for leaf, stem and flower fractions of five grass species.

	Leaf	**Stem**	**Flower**
Yield of VFA (mmol/g)			
Perennial ryegrass	-	0.37	1.45
Tall fescue	2.07	1.39	2.28
Yorkshire fog	1.23	1.12	1.27
Phalaris	1.80	0.34	1.84
Paspalum	1.03	1.45	0.72
Acetate:propionate ratio			
Perennial ryegrass	-	1.6	3.0
Tall fescue	3.9	2.6	1.9
Yorkshire fog	5.3	3.7	5.1
Phalaris	4.8	5.6	3.7
Paspalum	5.1	4.0	6.4

Maximum rates of VFA production occurred in the first 12 hours of fermentation, after which rates declined for each fraction of grass species. Net yield to 12 hours was similar for leaf and flower DM (Table 3) and usually lower for stems. Yields from ryegrass and phalaris stem were very low. The ratio of acetate:propionate did not show consistent patterns for leaf, stem and flower fractions across all species, with fog, paspalum and phalaris having higher proportions of acetate than ryegrass and fescue.

DISCUSSION

The primary purpose of this work was to establish the limits to digestion bought about by grass maturation. The data are intended to complement the digestion kinetic data determined by Barrell *et al*. (2000) and Burke *et al*. (2000) who established the mincing technique used in this study and described digestion kinetics of leafy material from immature, high-quality grasses and other forage types. Although the decline in nutritive value of mature grass is well known (Wilson, 1993), this decline will be a consequence of both changing composition of leaf and the development of stem and inflorescence. Data presented here confirms the slow degradation of stem, but also suggests a relatively low nutritive value of the flower, despite moderate concentrations of soluble carbohydrates in perennial ryegrass and tall fescue. In contrast, leaf from ryegrass and fog had high DM degradation rates (k=0.105 and 0.113) which were similar to values reported by Burke *et al.* (2000) for respective species (k=0.114 and 0.092). The chemical composition of young and old ryegrass and fog leaves were also similar (Table 1 and Burke *et al*., 2000) in contrast to tall fecue and paspalum leaves which contained substantially more NDF and less soluble carbohydrate when mature. Reductions in nutritive value appear to be primarily a consequence of decreasing proportion of leaf and increasing proportion of stem and flower, but composition of leaf appears to change with advancing maturity in some, but not all, species (Burke *et al*., 2000).

In general, leaf was more rapidly digested than stem and flower fractions, but with phalaris and paspalum the difference among all three constituents was relatively minor. *In vitro* incubations revealed insufficient nitrogen in paspalum and fescue leaves (and stem and inflorescence of all species) for sustained microbial growth, as evidenced by the very low ammonia concentration following 12 hours of incubations. Volatile fatty acid concentrations also showed lower quantities of acetate but increasing amounts of propionate after 12 hours in some incubations (data not presented), suggesting an alteration in microbial populations (Russell & Strobel, 1993), perhaps bought about by insufficient ammonia.

The principal factor affecting nutritive value of mature forages is the slow rate of degradation and clearance from the rumen of animals unable to select leafy material from the sward. A requirement to eat less digestible stem and flower is likely to restrict feed intake, and intake will be affected by the extent to which animals chew and reduce particle size of the forage during eating and rumination. An effective particle size reduction will facilitate clearance of slowly digested residues from the rumen. The mincer used to prepare these forages did achieve a particle size distribution similar to that entering the rumen of cattle fed fresh ryegrass and lucerne (Waghorn *et al*., 1989). However, measurements should be made of the particle size distribution in the swallowed *bolii* of sheep and cattle fed mature forages, C4 grasses or stalky material to confirm the validity of values obtained in this study for soluble (A), insoluble degradable (B) and effective digestibility (E) values predicted from *in sacco* incubations (Table 3). Despite this requirement for validation, comparative between particle size distribution for leaf, stem and inflorescence suggest significant differences in their nutritive value for ruminants, although the extent of these differences was not consistent across all grasses examined here.

Simulation models (e.g. Cornell Model (CNCPS), Pitt *et al*., 1996) are available for estimating nutrient supply to dairy cows, but rely on kinetic parameters of rumen digestion as inputs. These data are valuable as they provide information relating to mature forages, which are often major components for New Zealand dairy cows after peak lactation. The model outputs may assist in defining limitations to feed intake, digestion and nutrient release associated with pasture maturation. Identification of limitations will be used in developing feeding strategies to minimise post-peak lactation decline in milk yield in New Zealand dairying systems.

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