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Formulating total mixed rations from forages – defining the digestion kinetics of contrasting species

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ABSTRACT

The work described here is the first step in formulating forage-based TMR rations for dairy cows, based on digestion kinetics derived from *in vitro* and *in sacco* incubations. Kinetic information has been obtained from 23 contrasting fresh and conserved forages species, including temperate and tropical grass species, legumes (including Lotus spp. containing condensed tannins), herbs, silages, lucerne hay and maize grain. All forages were minced to a particle size similar to chewed material. Estimates of DM solubility (% DM) and fractional dry matter degradation rates (h^{-1}) determined by *in sacco* incubations include: white clover (39%, 0.195), perennial ryegrass (45%, 0.114), tall fescue (46.8%, 0.051), paspalum (27%, 0.068), sulla (51.4%, 0.121), chicory (41.8%, 0.260), pasture silage (41%, 0.083), maize silage (28%, 0.042). *In vitro* incubations resulted in five-fold differences between forages in net ammonia release, 2-3 fold differences in volatile fatty acid yield and a three-fold difference in acetate:propionate ratio. The data will be used in simulation models to identify optimal forage mixtures for meeting the nutritional needs of high-producing dairy cows.

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

INTRODUCTION

The New Zealand dairy industry is a low-cost pasture-based system, but the ryegrass-dominant diet has nutritional constraints that are limiting animal performance. However, there are alternative fresh and conserved forages able to be grown in New Zealand which have nutritive characteristics able to improve animal performance and welfare. This has been demonstrated in cattle and sheep fed legume-based diets, such as white clover (high protein and low fibre content) and *Lotus corniculatus*, *Lotus pedunculatus* and *Hedysarum coronarium* (which contain condensed tannins (CT)) in comparison with perennial-ryegrass-dominant diets (Ulyatt, 1981; Brown, 1990; Woodward *et al.*, 1998).

In North America, where concentrates are relatively inexpensive, nutrient balancing is used to formulate total mixed rations (TMR) for high-producing dairy cows. The same approach could be adopted in New Zealand using combinations of available forages with diverse chemical and structural characteristics. However, there is insufficient information concerning the digestion kinetics of fresh forages to formulate forage-based TMR.

Several methods are available for predicting nutritive value, each with their own advantages and disadvantages. Conventional feed evaluation to obtain estimates of digestibility, together with performance, is expensive and labour intensive. Chemical analyses of feeds determine nutrient composition (e.g., Corson *et al.*, 1999), but do not predict animal performance. Alternative procedures for estimating nutritive value and digestion kinetics involve incubating minced fresh forage *in vitro* (with rumen innoculum) and *in sacco* (forages placed in porous bags in the rumen). These two procedures enable digestion to be evaluated in terms of both products of digestion in vitro (ammonia and volatile fatty acids (VFA)) and rates of disappearance *in sacco*. In combination, these methods provide good information about the kinetics of digestion and the nutritive value of feeds.

The objective of this study was to define the digestion and fermentation kinetics of a diverse range of fresh and conserved forages to formulate a forage-based TMR best able to meet the nutritional requirements of high-producing dairy cows in New Zealand.

METHOD

Digestion kinetics were measured in 23 contrasting fresh and conserved forages comprising eight species of grass, five legumes (including Lotus spp.), two herbs, five types of silages, lucerne hay and maize grain (Table 1).

Fresh forages were collected by harvesting the top leafy horizon of each sward in a vegetative state, taking care to obtain representative samples of conserved forages. All material was frozen immediately following collection and maintained frozen until used in incubations.

Frozen material was chopped to about 30 mm to facilitate mincing (whilst frozen) in a Kreft Compact meat mincer R70 (Kreft, Gmbh), which resulted in a particle size distribution of the DM similar to chewed forages (Barrell *et al.*, 2000). The frozen minced material was either sealed into dacron bags for in sacco incubation, or weighed into bottles for in vitro incubation. Samples of minced forage were retained for chemical analysis by Near Infrared Reflectance Spectroscopy (NIRS), measurement of dry matter (DM) content and particle size distribution by wet sieving (Waghorn, 1986).

In sacco and *in vitro* incubations were carried out simultaneously, with three forages evaluated during each incubation. One ruminally cannulated non-lactating Holstein-Friesian cow fed good quality lucerne hay was used for all incubations, because previous experience had shown substantial variation between cows in digestion kinetics (Weimer *et al.*, 1999).

In sacco

About 30 g of minced forage (approximately 6 g DM) was placed in each 100 x 100 mm dacron bag (mean pore size 35 μm). Twelve bags of each forage were placed in

the rumen and duplicate bags removed at 0, 2, 6, 12, 24 and 72 h. Immediately after removal from the rumen, bags were hand-rinsed with cold water until no further colour appeared. A duplicate sample of fresh, minced perennial ryegrass was included as a standard and removed after 12h to monitor variation between runs. Bags were dried at 60°C for 48 hours and residues removed for analysis. Residues and forages were analysed by NIRS to estimate crude protein (CP), soluble carbohydrate and fibre contents.

Kinetic parameters of DM disappearance over time were predicted by fitting data from bag residues to a non-linear model (Ørskov and McDonald, 1979) using the Marquardt procedure (SAS, 1989-1996) for each forage. The model for digestion (e.g., Potential DM degradation (P_{DM})) was: $P_{DM} = A + B\ (1-e^{-k\ (t-L)})$ where A = soluble DM (%DM), B = degradable insoluble (%DM), k = fractional disappearance rate per hour ($\%h^{-1}$), t = incubation time (h) and L = lag time (h).

In vitro

About 2.5 g of freshly minced forage (approximately 0.5g DM) was weighed into 50 ml bottles and warmed to 39°C with 12 ml of buffer, 0.5 ml of reducing agent and 3 ml of strained rumen liquor as described by Barrell *et al.* (2000). Bottles were placed in a shaking incubator (90 oscillations $minute^{-1}$) for the duration of the incubation.

Triplicate bottles of each forage were removed after 0, 2, 4, 6, 8, 10, 12 and 24 h of incubation. The media and rumen liquor were subsampled for determination of ammonia, VFA and pH. A lucerne standard was included in each incubation for adjustment of between-incubation variation. Ammonia concentration was determined by the method of Chaney and Marbach (1962), and VFA based on the gas liquid chromatography method described by Attwood *et al.* (1998).

RESULTS

The chemical compositions of the forages are summarised in Table 1. Dry matter of fresh forages ranged from 11.6% to 30.9%. Composition of the DM was wide ranging with CP contents from 7.6% to 29.9%, soluble carbohydrate from 3.1% to 41.7% and total fibre content from 22.4% to 57.8%. Of the eight grasses evaluated, paspalum had the lowest soluble carbohydrate (4.2% DM) and CP (13.5% DM), and highest total fibre (57.8% DM) and consequently had the lowest predictable organic matter digestibility (64.9%). Legumes and herbs (chicory and plantain) had low fibre concentrations compared with most of the grasses and silages, and high crude protein contents ranging from 19.3% DM to 29.9% DM. Silages generally had low soluble carbohydrate contents ranging from 3.1% DM to 8.0% DM, indicating the conversion of sugars to lactic acid during ensilation.

The differences in chemical composition of forages, especially fibre content, were supported by the particle size distribution of minced material. Highly fibrous material (e.g., paspalum, lucerne silage and lucerne hay) had up to 56% of DM retained on sieves with aperture sizes larger than 1 mm, compared to as little as 28%, with plantain, white clover and chicory. Conversely the succulent forages had up to 37% of DM solubilised by mincing, compared to fibrous forages for which as little as 19% of DM was solubilised by mincing.

In sacco

In sacco DM digestion kinetics of the 23 forages are summarised in Table 2. Fractional dry matter degradation rates ranged from 0.051 h^{-1} (tall fescue) to 0.260 h^{-1} (chicory); and in the case of silages, from 0.042 h^{-1} (maize) to 0.167 h^{-1} (lucerne; Table 2). Forages with low concentrations of fibre in the DM lost 94-95 % of DM to digestion over 72 h, compared with fibrous or conserved forages where losses were 83% (paspalum), 82% (maize silage) and 74% (lucerne hay). Figure 1 illustrates the DM degradation curves for nine contrasting feeds evaluated. The period during which either no digestion occurs, or digestion occurs at a greatly reduced rate, is generally referred to as the lag phase (McDonald, 1981). In this study the duration of the lag phase ranged from 0 h (sulla, lucerne hay and sulla silage) to 7.5 h (kikuyu).

In vitro

In vitro incubation enabled the products of digestion to be determined (Figure 2). Under normal grazing situations, forage DM will be resident in the rumen for about 6-24 h, and after 6 h of *in vitro* incubation there was a five-fold difference between forages in the proportion of plant nitrogen (N) released to ammonia-N (paspalum, 0.040 vs. Yorkshire fog, 0.217). The net yield of ammonia showed as much as 52% of plant-N was released over 24 h (lucerne; Figure 2), but forages with low N content (e.g., maize grain, maize silage and paspalum) showed a maximum net yield of ammonia after 6 h, followed by a net loss after 24 h. Net losses suggest N incorporation into microbial biomass exceed N release from plant protein degradation. Forages containing CT (Lotus spp. and sulla) released 0.10-0.25 of plant N to ammonia (Figure 2), despite relatively high N concentrations in the DM (21.5 – 23.0%), but ensiling sulla increased ammonia yield about two-fold relative to fresh sulla.

Yields of VFA showed a 2.5-fold range across forages and although red clover had the highest yields throughout the 24-h incubation there were substantial differences between forages in the rates of VFA production over 24 h. After 24 h of incubation, red clover, Tama ryegrass and lucerne had produced the greatest amount of VFA and plantain, kikuyu and maize grain the least. The ratio of acetate to propionate (A:P ratio) also showed a substantial difference between forage types. Tama ryegrass, perennial ryegrass, white clover and sulla had the lowest A:P ratios after 6 h (1.7, 2.1, 2.2, 2.2; respectively) and after 24 h, sulla and white clover continued to have the lowest A:P ratios (2.2 and 2.3, respectively). In contrast, plantain and maize silage had the highest A:P ratios after 6 h (5.9 and 5.0, respectively), although the ratios declined to 2.5 and 3.2 for the respective forages after 24 hours.

DISCUSSION

This study has produced the first comprehensive set of degradation data for forages prepared in a manner similar to that consumed by ruminants. The combined use of *in sacco* and *in vitro* techniques indicate actual losses through

TABLE 1. Forage dry matter (DM) content and composition (% of DM) and predicted organic matter digestibility (OMD) determined by Near Infrared Reflectance Spectroscopy for fresh and conserved species.

Forage	DM (%)	Soluble Carbohydrates	Crude Protein	Total Fibre[a]	OMD (%)
Fresh					
Lolium perenne (Perennial ryegrass)	18.8	9.1	15.5	48.7	77.3
Dactylis glomerata (Cocksfoot)	26.8	7.4	23.7	47.5	74.7
Festuca arundinacea (Tall fescue)	25.3	11.9	16.4	41.6	75.6
Holcus lanatus (Yorkshire fog)	16.3	12.3	23.7	39.9	85[b]
Bromus willdenowii (Prairie grass)	19.1	9.9	19.9	44.8	75.2
Lolium multiflorum (Grasslands Tama)	15.2	16.4	21.3	36.5	85[b]
Pennisetum clandestinum (Kikuyu)	17.2	6.7	16.4	47.7	65.9
Paspalum dilatatum (Paspalum)	30.9	4.2	13.5	57.8	64.9
Trifolium repens (White clover)	15.0	12.1	26.9	25.6	82.1
Lotus corniculatus (Birdsfoot trefoil)	16.2	13.0	22.2	28.2	76.9
Lotus pedunculatus	16.3	12.2	21.5	33.1	80.3
Medicago sativa (Lucerne)	23.9	8.6	29.9	29.5	73.0
Trifolium pratense (Red Clover)	14.8	8.5	27.4	33.6	85[b]
Hedysarum coronarium (Sulla)	11.6	17.8	23.0	22.4	85[b]
Cichorium intybus (Chicory)	14.3	11.4	19.3	23.8	83.9
Plantago lacelata (Plantain)	13.0	14.0	24.7	28.3	85[b]
Conserved					
Lucerne silage	57.4	8.0	23.3	30.5	-[c]
Maize silage	34.7	41.7	7.6	40.5	-[c]
Oat silage	40.0	3.1	17.8	53.2	-[c]
Pasture silage	40.8	3.7	17.2	50.3	-[c]
Sulla silage	22.6	4.1	21.2	36.2	-[c]
Lucerne hay	89.9	4.9	24.2	39.1	65.6
Zea maize (Maize grain)	87.1	71.0	10.2	10.9	

[a] Cellulose, hemicellulose and lignin
[b] Very high estimated digestibility, constrained to 85%
[c] OMD not predicted for silages by NIRS

TABLE 2. Forage dry matter (DM) degradation characteristics (% of DM) as defined by soluble DM (A), degradable insoluble fraction (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 from the rumen.

Forage	A	B	P	k	L	E[1]
Fresh						
Perennial ryegrass	45	49	94	0.114	4.6	74
Cocksfoot	49	43	92	0.120	4.7	75
Tall fescue	47	49	96	0.051	4.6	65
Yorkshire fog	49	46	95	0.092	4.2	73
Prairie grass	41	51	92	0.091	0.5	71
Tama ryegrass	55	42	97	0.098	4.6	78
Kikuyu	31	55	86	0.071	7.5	53
Paspalum	27	56	83	0.068	4.7	52
White clover	39	55	94	0.195	0.9	81
Lotus corniculatus	51	39	90	0.151	1.2	74
Lotus pedunculatus	43	42	85	0.108	4.5	67
Lucerne	49	39	89	0.131	0.4	76
Red clover	21	65	86	0.130	0.9	65
Sulla	51	44	95	0.121	0	81
Chicory	42	52	94	0.260	0.4	84
Plantain	38	54	92	0.246	0.9	81
Conserved						
Lucerne silage	47	37	84	0.167	4.1	72
Maize silage	28	54	82	0.042	0.6	50
Oat silage	42	46	91	0.070	1.5	69
Pasture silage	41	50	91	0.083	4.0	67
Sulla silage	49	40	89	0.059	0	69
Lucerne hay	28	47	74	0.073	0	53
Maize grain	17	77	94	0.066	0.3	58

[1] Calculated using an assumed fractional passage rate of 0.06 h^{-1}.
[2] Dairying Research Corporation (Ltd), Private Bag 3123, Hamilton, New Zealand.

FIGURE 1: Dry matter degradation curves of nine forages evaluated *in sacco*

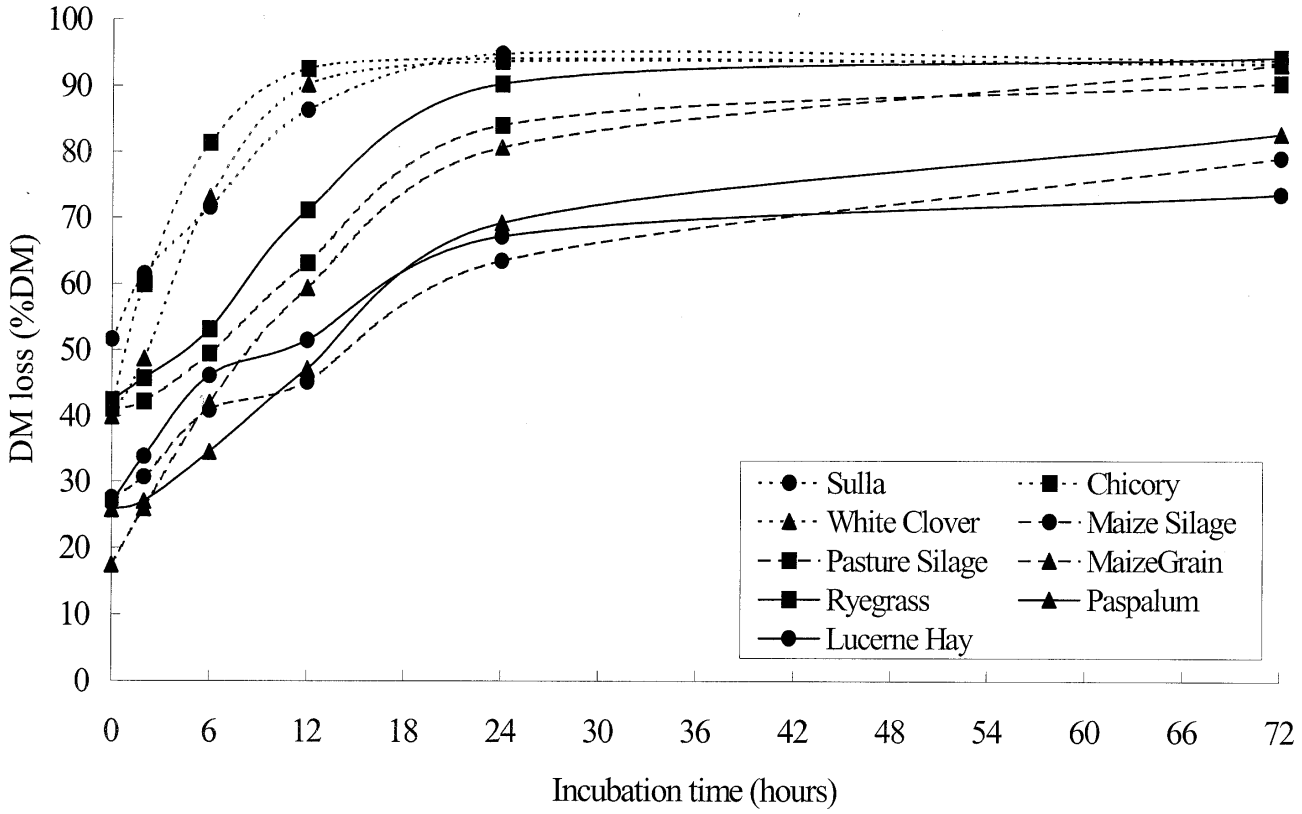
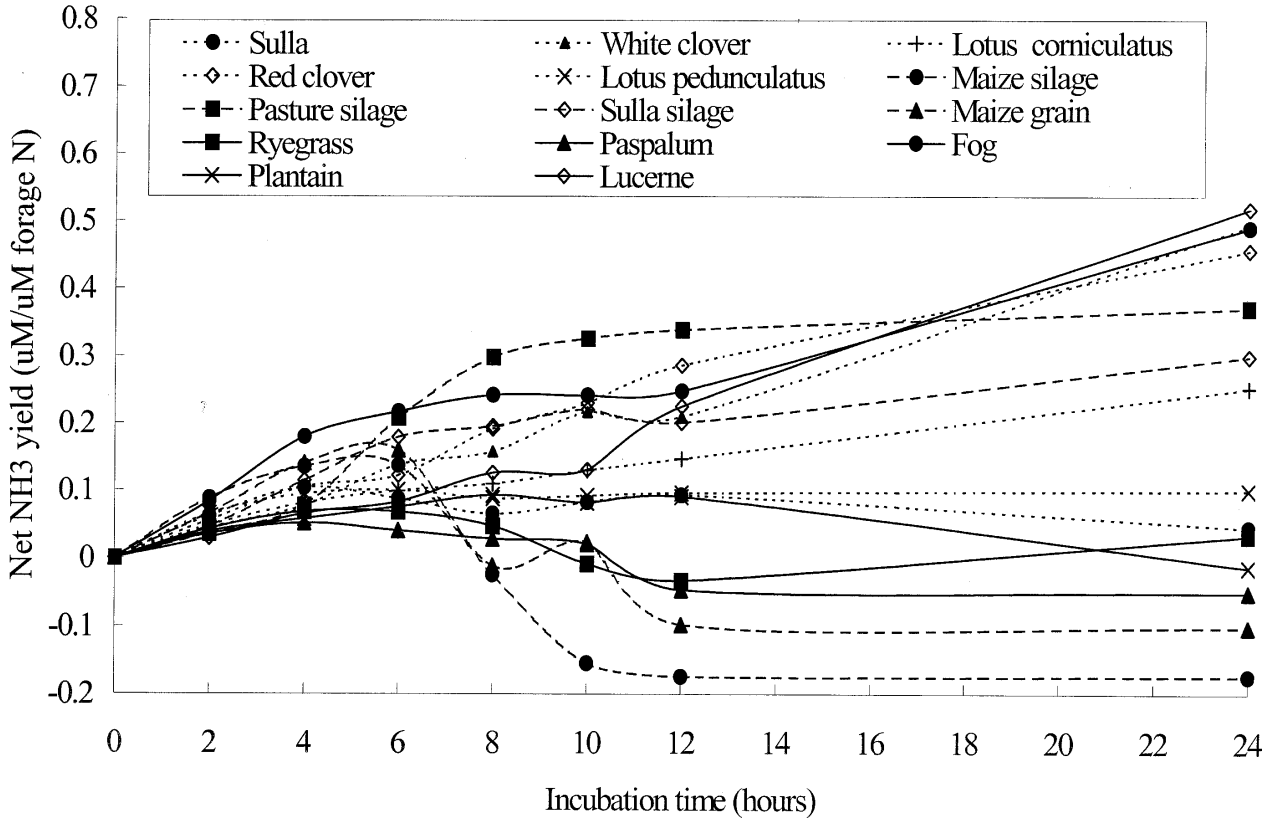


FIGURE 2: Net NH_3 yield for 14 forages evaluated *in vitro*



digestion in the rumen (*in sacco*), and the net yield of metabolites from fermentation (*in vitro*) that are absorbed and metabolised by ruminants.

An important component of these data was the use of fresh, minced forages to replicate chewed material characteristic of ruminant diets in New Zealand. Although incubations have been undertaken with fresh, chopped perennial ryegrass, cocksfoot, white clover and Lotus corniculatus (Van Vuuren *et al.*, 1991; Goplen *et al.*, 1992; Kolver *et al.*, 1998; Barrell *et al.*, 2000) most evaluations have been based on freeze-dried and ground preparations. The type of preparation has very significant effects on degradation kinetics, in terms of DM disappearance, proteolysis, VFA production and microbial growth.

The kinetic information corresponds to known differences in nutritive value. For example, the k value for perennial ryegrass (0.114 h^{-1}) was much lower than that for white clover (0.195 h^{-1}) and the initial degradation of perennial ryegrass was slow (L = 4.6 h) relative to white clover (L = 0.9 h). These differences are indicated by the effective degradability (E; Table 2) of the two feeds and are supported by the relatively poor performance of ruminants fed on perennial ryegrass relative to white clover (Ulyatt, 1981; Harris *et al.* 1997). The kinetics presented here were derived from lush, leafy material and differences would be greater with more mature forage typical of summer growing conditions (Clark and Brougham, 1979).

The 23 forages evaluated in this study include examples of slow degrading forages such as kikuyu and paspalum for which rates of DM loss were 0.071 h^{-1} and 0.068 h^{-1}, respectively with substantial lag times (4.5 and 7.5 h, respectively), compared to chicory and plantain for which rates of DM loss were 0.260 h^{-1} and 0.246 h^{-1}, respectively with short lag times (0.4 h and 0.9 h, respectively). These data demonstrate some of the reasons why animal performance can be very poor on some diets (slow degradation is related to slow rumen clearance and low intakes), but more importantly, enable forage-based diets to be formulated with a sound kinetic basis.

Production of ammonia *in vitro* indicates the extent of wasteful protein degradation that occurs during digestion. This study has shown that forages with high concentrations of crude protein and low fibre concentrations (e.g., white clover and red clover) have a faster rate and greater extent of dry matter degradation and undergo more proteolysis than forages with low-crude-protein contents (e.g., maize silage and paspalum). Excess ammonia produced and not utilised by microbes has to be excreted as urea at a net metabolic cost to the animal. In contrast, forages with insufficient degradable protein (e.g., paspalum and maize silage) have a high utilisation (low ammonia production) which may limit microbial activity, fibre degradation and subsequent yield of VFA. Forages with contrasting crude protein availability may be complementary and result in a more efficient protein and energy capture when fed as a mixed diet than either fed alone.

VFA represent about 65-75% of energy available to ruminants and the rates of production and ratios of the usually dominant acetate (60-70% of VFA from pasture) to the glucogenic propionate, has important implications for nutrient supply. For example, milk yield is dependent upon a supply of glucose, much of which will be derived from propionate.

In vitro incubations have produced some interesting insights into feed value. For example, red clover, which was once an important component of ruminant diets has a very high nutritive value and could be given more prominence in diet formulations (perhaps with sulla). In contrast, plantain digestion is rapid *in sacco*, but minimal *in vitro*, suggesting anti-microbial compounds might be present.

The data presented here are complex and extensive and require kinetic simulation models to balance nutrient yields with dairy cow requirements. Animal nutrient requirements are well-established (AFRC, 1993; NRC, 1989), and the information derived from these incubations will provide a scientific basis for formulating forage-based rations. Forages used in formulations will complement each other in terms of their digestion and fermentation characteristics and optimise the nutrient supply for high-producing dairy cows in New Zealand.

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