In vitro production of volatile fatty acids from forages

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ABSTRACT

Volatile fatty acid (VFA) production from a range of fresh forages was measured *in vitro* over 24 hours (h). The forages comprised temperate and tropical grasses, legumes (including some containing condensed tannins; CT), herbs and silages, all of which were minced to resemble chewed forage and incubated in buffered media. Volatile fatty acid concentrations were measured in triplicate for each feed at 0, 6, 12 and 24 h. On average, VFA yields were 131, 226 and 311 mg/g dry matter (DM) between 0 – 6, 0 – 12 and 0 – 24 h, and the highest yields were from legumes (384 mg/g DM) and lowest from plantain (176 mg/g DM) after 24 h. Legumes containing CT produced 28% less VFA than other legumes. The acetate + butyrate:propionate (A+B:P) ratio showed a substantial difference between forage types, ranging from an average of 2.5 (legumes) to 3.5 (tropical grasses) after 6 h, but differences between forage types diminished after 24 h. Multiple regression analysis showed a weak inverse relationship with NDF (neutral detergent fibre) after 6 h, but there was no clear relationship with particle size, chemical composition or digestion rates at 12 and 24 h.

Keywords: *In vitro* digestion; volatile fatty acids; forages

INTRODUCTION

New Zealand livestock farming relies on ryegrass-based pasture, but ryegrass has nutritional constraints that can limit production. Sheep and cattle fed legume-based diets, such as *Trifolium repens* (white clover), *Lotus corniculatus* (birdsfoot trefoil), *Lotus pedunculatus* (lotus) and *Hedysarum coronarium* (sulla) have achieved better liveweight gains and milk production compared to ryegrass-based pasture (Ulyatt, 1981; Brown, 1990; Terrill *et al*., 1992; Woodward *et al*., 1999). Some of the responses are due to intake, but the legumes can also increase the efficiency of nutrient utilisation (Burke, 2004; Waghorn and Barry, 1987).

Volatile fatty acids (VFAs) represent a major product of ruminant digestion, account for 70-80% of absorbed energy and their proportions affect animal production. For example, high proportions of propionate can cause milk fat depression but when acetate supply exceeds the energy needs it is stored as fat. Although nutritive value (NV) is indicated by forage chemical composition, digestion by the rumen microflora affects the composition of nutrients absorbed by the host. Microbial activity is regulated to some extent by the host animal (Weimer *et al*, 1999) and is affected by substrate availability, especially DM release from ruptured plant cells and particulate surface area available for bacterial colonisation.

Rather than undertaking feeding trials with sheep or cattle to evaluate forages fed as monocultures or mixtures, ration balancing programmes similar to those used in North America, would enable mixtures to be formulated to meet ruminant needs for defined levels of production. The data presented here contribute information needed to predict animal performance from contrasting forages.

The objective of this research was to measure the quantity and proportions of VFAs produced from contrasting forages after 6, 12 and 24 hours (h) using *in vitro* incubations. These periods represent typical rumen residence times for liquid and solid fractions and most *in vivo* digestion would have taken place after 24 h. The data will assist in the prediction of animal production.

MATERIALS AND METHODS

Digestion kinetics using *in vitro* techniques were measured with 22 contrasting fresh (frozen) and conserved forages comprising six species of temperate grasses (perennial ryegrass, cocksfoot, tall fescue, yorkshire fog, prairie grass and annual ryegrass), two tropical grasses (kikuyu and paspalum), six legumes (white clover, red clover, lucerne, birdsfoot trefoil, lotus and sulla) and two herbs (chicory and plantain). The silages were pasture, oat, maize, lucerne and sulla and lucerne hay was also evaluated. The botanical names of the forages are listed in Table 1.

Leaf samples of fresh grasses and herbs, and leaves with stems or petioles of legumes were collected while plants were in the vegetative state. Samples of conserved forages were also collected and all material was frozen immediately following

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collection and maintained frozen until incubated (Burke *et al*., 2000).

Frozen material was minced with a Kreft Compact meat mincer, so that the forage had a particle size distribution similar to herbage material chewed by ruminants (Burke *et al*., 2000). Samples of minced forage were used for determining DM content, predicted nutrient composition using Near Infrared Reflectance Spectroscopy (NIRS) and particle size distribution by wet sieving (Waghorn, 1986). In addition to the *in vitro* incubations reported here *in sacco* incubations had been undertaken (Burke, 2004) to indicate the distribution of fibre and protein between soluble (A) and insoluble degradable (B) fractions.

In vitro incubation

About 2.5 g of freshly minced forage (approximately 0.5g DM) was weighed into 50 ml vented bottles and warmed to 39^{o}C with 12 ml of McDougall's buffer, 0.5 ml of reducing agent and 3 ml of strained rumen liquor as described by Burke *et al*. (2000). Rumen inocula for all incubations were obtained from one rumen cannulated non-lactating Holstein-Friesian cow that was fed good-quality lucerne hay. Bottles were placed into a shaking incubator (90 oscillations/minute) for the incubation.

Triplicate bottles of each forage were removed after 0, 2, 4, 6, 8, 10, 12 and 24 h of incubation. The contents were analysed for pH and ammonia concentration, which have been reported in Burke *et al*. (2000). At 6, 12 and 24 h, 1.5 ml samples were bulked from triplicates within feeds and VFA concentrations determined using gas liquid chromatography (Attwood *et al*., 1998) and expressed as mg VFA/g DM incubated.

Statistical Analysis

Multiple regression analyses were used to determine the relationship between yields and A+B:P ratios of the VFA after 6, 12 and 24 h with diet composition, particle size of minced forage and degradation kinetics determined from *in sacco* incubations of the same samples (Burke *et al*., 2000). The stepwise procedure of SAS (1996) was used to generate regression relationships to identify variables most likely to affect VFA production. The level of significance was set at $P < 0.10$ in order to prevent the selection of too many variables in the multiple regression models. Variables included in the model comprised three chemical constituents (crude protein, CP; neutral detergent fibre, NDF; non fibrous carbohydrates, NFC), two digestion rates (CP and NDF) with lags, and three particle size fractions (soluble, < 0.5 mm and > 0.5 mm particles).

RESULTS

The fresh forages varied from 12 to 31% dry matter (DM), and contained 14 to 30% CP, 22 to 58% NDF and 14 to 37% NFC (calculated as 100 - (CP + NDF + lipid + ash) (Table 1). The conserved feeds were even more contrasting, with maize silage containing only 8% CP, 41% NDF and 43% NFC compared with pasture silage which contained more CP (17%) and NDF (50%) and less NFC (15%). Generally legumes contained more

TABLE 1: Dry matter (DM) concentration, chemical composition (% of DM) and metabolisable energy (ME) of fresh and conserved forages used for *in vitro* incubations.

Forage	Forage type	DM (%)	Non-fibrous carbohydrat	Crude Protein	Neutral detergent fibre	Ash	ME (MJME/kgD
Lolium perenne (Perennial ryegrass)	Temperate	19	21	16	49	11	10.9
Dactylis glomerata (Cocksfoot)	Temperate	27	14	24	48	10	11.1
Festuca arundinacea (Tall fescue)	Temperate	25	27	16	42	12	11.3
Holcus lanatus (Yorkshire fog)	Temperate	16	22	24	40	11	12.7
Bromus willdenowii (Prairie grass)	Temperate	19	20	20	45	12	11.2
Lolium multiflorum (Grasslands Tama)	Temperate	15	27	21	37	11	12.7
Pennisetum clandestinum (Kikuyu)	Tropical	17	20	16	48	12	9.8
Paspalum dilatatum (Paspalum)	Tropical	31	16	14	58	10	9.7
Trifolium repens (White clover)	Legume	15	31	27	26	13	11.5
Medicago sativa (Lucerne)	Legume	24	27	30	30	10	10.9
Trifolium pratense (Red Clover)	Legume	15	22	27	34	13	11.1
Lotus corniculatus (Birdsfoot trefoil)	Legume, (CT)[1]	16	33	22	28	9	11.0
Lotus pedunculatus (Lotus)	Legume, (CT)[1]	16	27	22	33	10	12.0
Hedysarum coronarium (Sulla)	Legume, (CT)[1]	12	33	23	22	13	12.7
Cichorium intybus (Chicory)	Herb	14	37	19	24	17	12.5
Plantago lanceolata (Plantain)	Herb	13	27	25	28	16	11.7
Pasture silage	Conserved	41	15	17	50	13	11.0
Oat silage	Conserved	40	7	18	53	17	11.1
Lucerne silage	Conserved	57	30	23	31	12	11.2
Sulla silage	Conserved, (CT)[1]	23	23	21	36	12	11.5
Maize silage	Conserved	35	43	8	41	5	10.7
Lucerne hay	Conserved	90	24	24	39	10	9.8

[1] These legumes contained condensed tannin (CT) in the DM: *Lotus corniculatus* = 3.1%; *Lotus pedunculatus* = 5.1%; Sulla = 5.3%; Sulla silage = 1.1%.

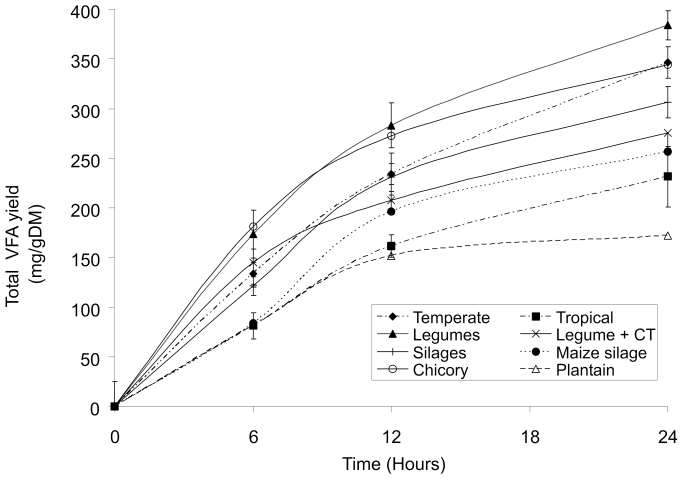
protein and less fibre and had lower DM concentrations than temperate grasses, and birdsfoot trefoil, lotus and sulla contained condensed tannins (CT). Chicory and plantain are classified as herbs and contained low concentrations of fibre and relatively high concentrations of CP and NFC. Tropical grasses had relatively high fibre and low protein concentrations and low predicted metabolisable energy (ME).

Particle size distribution varied substantially between forages (Table 2). Tropical grasses had less soluble and residual DM (< 0.25 mm; 26 to 33%), than legumes (31 to 50% of DM), temperate grasses (36 to 54% of DM) and herbs (38 and 44% DM). Larger particles (> 0.5 mm) accounted for over 40% of DM from tropical grasses and 30 to 39% of DM for temperate grasses. The proportions of large particles varied considerably for legumes (27 to 45%), probably as a consequence of leaf and stem proportions and the ease of mincing. Tough material (eg. grasses and silages) took longer to mince compared to legumes, so differences in particle size between forage types was less than indicated by chemical composition.

In vitro pH and VFA Production

In vitro pH was used to monitor incubations. At the start of the incubation pH was between 7.0 and 7.4 and after 12 h ranged between 5.9 to 7.2. When values dropped to 5.6 or below, the environment in the bottle was considered to be unrepresentative of normal digestion, but this only occurred at 24 h with three temperate grasses and maize silage.

FIGURE 1: Net yield of volatile fatty acids (VFA; mg/g DM) produced when forages[1] were evaluated *in vitro*. Standard error bars for temperate and tropical grasses, legumes with and without condensed tannin (CT) and silages.



Yields of VFAs (Table 2) showed that production was greatest for legumes over 24 h, and least for tropical grasses (Figure 1). On average, the legumes containing CT produced less VFA (275 mg/gDM) during the entire 24 h, compared to legumes without CT (384 mg/gDM) (Figure 1). At each sampling time, there was a two-fold difference in total VFA yields with highest values from red clover at 6, 12 and 24 h (218, 327 and 407 mg/g DM, respectively). Paspalum produced the least amount of VFAs at 6 and 12 h (68 and 150 mg/gDM, respectively), but at 24 h plantain had the lowest yield (172 mg/g DM), despite a very low

TABLE 2: Particle size distribution of minced forages used for *in vitro* incubtaions, pH after 12 hours (h) and yields and molar ratios of volatile fatty acids (VFA) after 6, 12 and 24 h.

	Particle size (mm)			*In vitro* pH	Net VFA yield (mg/g DM)			A+B:P ratio[1]		
	> 0.5	0.5 – 0.25	< 0.25	12 h	6 h	12 h	24 h	6 h	12 h	24 h
Fresh										
Perennial ryegrass	38.8	15.5	45.7	5.9	130	212	287	2.3	2.4	2.8
Cocksfoot	33.2	31.0	35.8	6.3	116	166	328	3.5	3.1	2.8
Tall fescue	34.2	17.4	48.4	6.3	158	229	353	3.2	3.3	3.4
Yorkshire fog	36.8	13.4	49.8	6.5	109	220	339	3.5	3.2	3.2
Prairie grass	30.1	18.8	51.1	6.3	117	255	376	3.4	3.5	3.6
Grasslands Tama	35.9	9.7	53.9	6.3	174	320	398	2.3	2.8	3.0
Kikuyu	41.4	25.6	33.0	6.9	95	173	201	2.8	3.1	3.0
Paspalum	46.0	28.5	25.5	6.7	68	150	262	4.2	3.7	3.0
White clover	41.5	20.7	37.7	6.8	170	251	356	2.2	2.1	2.6
Lucerne	38.2	17.6	44.3	6.4	134	271	388	2.1	2.4	2.7
Red Clover	44.6	24.3	31.1	7.0	218	327	407	3.1	3.8	3.9
Birdsfoot trefoil	32.0	18.4	49.6	6.9	108	212	317	2.3	2.2	2.7
Lotus	27.3	29.8	42.9	6.6	125	178	240	3.3	4.0	4.5
Sulla	30.5	23.4	46.2	7.2	201	233	268	2.2	2.5	2.5
Chicory	35.9	20.0	44.2	6.8	182	273	344	3.2	3.8	4.0
Plantain	27.6	34.0	38.4	7.0	81	152	172	2.1	2.9	2.7
Conserved										
Pasture silage	39.0	17.6	43.4	6.6	108	210	305	2.4	2.8	2.9
Oat silage	42.3	12.6	45.1	6.7	121	226	328	2.9	3.4	3.4
Lucerne silage	54.8	15.5	29.8	5.9	143	245	284	2.6	2.1	2.4
Sulla silage	31.1	22.4	46.5	6.5	150	276	357	2.3	2.7	3.0
Maize silage	33.2	26.0	40.9	6.2	84	196	257	5.0	4.6	3.7
Lucerne hay	56.3	17.5	26.3	6.7	96	199	277	3.8	3.8	3.7

[1] A+B:P, molar ratios of acetate + butyrate:propionate

fibre content. For white clover, red clover and lucerne, the VFA yield was equivalent to 36 to 40% of the DM, while for temperate ryegrasses it was equivalent to 29 to 38% of the DM. Generally, the rate of production for all forages was fastest for the first 6 h of incubation and declined thereafter (Figure 1; Table 2).

Forages produced 45 to 55% acetate, 20 to 25% propionate and 15 to 20% butyrate. The range in A+B:P ratios between forage types was greatest after 6 h (2.5 – 3.5) with highest values for tropical grasses and the lowest ratio for legumes (Table 2). By 24 h the proportions of propionate had decreased and the differences between forage types ranged from 3.0 to 3.4. There was little change in the average ratio for temperate grasses during the 24 h incubation, but for legumes the ratio increased from 2.5 to 3.1. There were also differences between forages within groups (Table 2), for example lotus had a higher A+B:P ratio after 24 h (4.5) than birdsfoot trefoil (2.7) and sulla (2.5).

DISCUSSION

The VFA data presented here complements *in vitro* ammonia yields and *in sacco* DM degradation kinetics reported by Burke *et al* (2000). *In vitro* incubations indicate net yields from fermentation (ammonia and VFA), while *in sacco* incubations measure actual losses through digestion in the rumen. Together, these results provide a comprehensive data-set defining digestion and fermentation of a range of fresh forages and enables ranking in terms of yield and glucogenic potential of the VFAs.

An important aspect of this work was the use of fresh forages prepared by mincing, rather than freeze-drying, grinding or chopping (Van Vuuren *et al*., 1991; Goplen *et al*., 1993; Kolver *et al*., 1998; Barrell *et al*., 2000). The type of preparation has significant effects on degradation kinetics, VFA production and microbial growth (Barrell *et al*., 2000) so the particle size distribution has been included in the multiple regression analysis to find factors likely to affect VFA production. Mincing the fresh forage was intended to replicate chewed material.

Volatile fatty acids represent about 70 – 80% of energy absorbed by ruminants and both rates of production and proportions of individual VFAs affect energy supply as well as glucogenesis and milk production (Waghorn and Barry, 1987). Highest VFA yields were obtained from legumes and chicory (Figure 1) all of which contained a low NDF concentration, whilst lowest yields originated from more fibrous forages, especially the tropical grasses (Table 2). Overall VFA yields after 24 h of incubation represented 17 – 41% of DM incubated. The very low value for plantain suggests microbial inhibition, especially as the chemical composition would indicate a very rapid fermentation.

Although acetate plus butyrate typically account for 75% or more of the VFAs produced from pasture digestion by cows (Church, 1976; Bergman, 1990; Mackle *et al*., 1996), the 12 h *in vitro* data suggest a considerable range between species, from as little as 64% of VFA (white clover) to 80% (maize silage). Comparable values after 24 h of incubation were 66% (sulla and lucerne) to 79% (lotus) with a mean of 71%. A higher proportion of propionate results in increased energy capture relative to acetate (Waghorn and Barry, 1987) so forages with a low A+B:P ratio (e.g. 2.1-2.2; white clover, birdsfoot trefoil at 12 h) should have a higher nutritive value than those with higher ratios (e.g. lotus, maize silage; Table 2).

The multiple regression analysis of VFA production demonstrated a weak negative effect of forage NDF concentration on VFA yield after 6 h (r^2 = 0.42), but no clear effects of particle size distribution, chemical composition, or *in sacco* digestion rates on VFA production were evident at 12 and 24 h. The ratios of A+B:P ratio were not clearly affected by any parameter or interaction tested and other factors must have affected the microbial populations and products of fermentation.

The *in vitro* incubations have demonstrated substantial variations in products of digestion from contrasting forages. The importance of VFA yields and proportions for efficient animal production have been indicated and the merits of legumes, including some with CT are well known (Terrill *et al*., 1992; Woodward *et al*., 1999; Burke *et al*., 2002), but the challenge is how to make best use of these diets for diet formulation and feeding systems. Inclusion of digestion rates and products into simulation models will provide a sound scientific basis for improving the nutrition of ruminants with high genetic potential for production. This is likely to become increasingly important as pasture clover contents are reduced and tropical grasses increase in some regions.

CONCLUSIONS

The data presented here provide a ranking of grasses, legumes, herbs and silages in terms of their production of VFAs during *in vitro* fermentation. Differences between fermentation and degradation characteristics of a variety of forages are supported by production differences observed in animal studies. These data may be used in conjunction with nutrient balancing models

to develop forage mixtures to improve animal performance.

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REFERENCES

Attwood, G.T.; Klieve, A.V.; Ouwerkerk, D.; Patel, B.K.C. 1998. Ammonia-hyperproducing bacteria from New Zealand ruminants. *Applied and Environmental Microbiology* 6-5: 1796-1804.

Barrell, L.G.; Burke, J.L.; Attwood, G.T.; Brookes, I.M.; Waghorn, G.C. 2000. Preparation of fresh forages for incubation and prediction of nutritive value. *Proceedings of the New Zealand Society of Animal Production* 60: 5-8.

Bergman, E.M. 1990. Energy contribution of VFA from the gastrointestinal tract in arious species. *Physiological Reviews* 70: 567-590.

Brown, C. 1990. An integrated herbage system for Southland and South Otago. *Proceedings of the New Zealand Grassland Association* 52: 119-122.

Burke, J.L. 2004. Determination of digestion parameters to develop and evaluate forage mixtures for pasture-fed ruminants: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Science at Massey University, Palmerston North, New Zealand.

Burke, J.L.; Waghorn, G.C.; Brooks, I.M. 2002. An evaluation of *sulla (Hedysarum coronarium*) with pasture, white clover and Lucerne for sheep. *Proceedings of the New Zealand Society of Animal Production* 62: 152-156.

Burke, J.L.; Waghorn, G.D.; Brookes, I.M.; Attwood, G.T.; Kolver, E.S. 2000. Formulating total mixed rations from forages – defining the digestion kinetics of contrasting species. *Proceedings of the New Zealand Society of Animal Production* 60: 9-14.

Church, D.C. 1976. Rumen fermentation of natural feedstuffs. In: *Digestive Physiology and Nutrition of Ruminants; Vol.1*, *Digestive physiology Second edition.* Pp. 280-311. O and B books, Corvallis, Oregon, USA.

Goplen, B.P.; Howarth, R.E.; Lees, G.L. 1993. Selection of alfalfa for a lower initial rate of digestion and corresponding changes in epidermal and mesophyll cell wall thickness. *Canadian Journal of Plant Science* 73: 111-122.

Kolver, E.S; Muller, L.D.; Varga, G.A.; Cassidy, T.J. 1998. Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. *Journal of Dairy Science* 81: 2017-2028.

Mackle, T.M.; Parr, C.R.; Bryant, A.M. 1996. Nitrogen fertiliser effects on milk yield and composition, pasture intake, nitrogen and energy partitioning, and rumen fermentation parameters of dairy cows in early lactation. *New Zealand Journal of Agriculture Research* 39: 341-356.

SAS. 1996. Version 6.12 Edition. The SAS System for Windows. SAS Institute Inc., Cary, North Carolina, USA.

Terrill, T.H.; Douglas, G.B.; Foote, A.G.; Purchas, R.W.; Wilson, G.F.; Barry, T.N. 1992. Effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. *Journal of Agricultural Science, Cambridge* 119: 265-273.

Ulyatt, M.J. 1981. The feeding value of herbage: Can it be improved? *New Zealand Agricultural Science*. 15-4: 200-205.

Van Vuuren, A.M.; Tamminga, S.; Ketelaar, R.S. 1991. *In sacco* degradation of organic matter and crude protein of fresh grass (*Lolium perenne*) in the rumen of grazing dairy cows. *Journal of Agricultural Science, Cambridge* 116: 429-436.

Waghorn, G.C. 1986. Changes in rumen digesta of cows during a restricted eating period when fed fresh red clover, lucerne or lucerne hay. *New Zealand Journal of Agricultural Research* 29: 233-241.

Waghorn, G.C.; Barry, T.N. 1987. Pasture as a nutrient source. In: *Livestock feeding on pasture. Occasional publication No. 10*. Ed. Nicol, A.M. Pp. 21-38. New Zealand Society of Animal Production, Hamilton, New Zealand.

Weimer, P.J.; Waghorn, G.C.; Odt, C.L.; Mertens, D.R. 1999. Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. *Journal of Dairy Science* 82: 122-134.

Woodward, S.L.; Auldist, M.J.; Laboyrie, P.J.; Jansen, E.B.L. 1999. Effect of *Lotus corniculatus* and condensed tannins on milk yield and milk composition of dairy cows. *Proceedings of the New Zealand Society of Animal Production* 59: 152-15.