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Digestion kinetics of ryegrass

A.V. CHAVES[1, 2], G.C. WAGHORN[1], I.M. BROOKES[2] AND D. HEDDERLEY[3]

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

ABSTRACT

Changes in ryegrass maturation, with accompanying decreases in nutritive value and feed intake, have significant consequences for the dairy industry. Thus digestion kinetics of ryegrass were measured in samples differing in both physiological age (days after cutting) and calendar date to form a database that can be used as a guide to calculate the extent and type of supplement needed to match nutrient supply with requirements of grazing dairy cattle. Ryegrass pasture was mown on 21 August, 11 September, and 21 September and samples of approximately 2 kg were harvested from those dates at 7-14 day intervals for chemical analyses by cutting to 5 cm above soil level. These samples were used for *in sacco* and *in vitro* incubations to determine rate of digestion and proteolysis. The principal finding was a rapid decline in crude protein content from about 22.5% of DM harvested at 21 days to 7.2% for that harvested after 67 days. These changes were associated with increases in the neutral detergent fibre (NDF) fraction of the DM (42.1% to 56.3%) and in lignin concentration (2.49% to 3.07%). Changes were more rapid in late-cut than early cut forages. The principal consequences of increased maturity were slower degradation rates of DM (k = 0.067 to 0.038/hour) and CP (k = 0.122 to 0.052/hour) and less degradation of crude protein to ammonia. This data can be used in conjunction with dairy cow models (e.g., CNCPS) to predict animal performance.

Keywords: ryegrass; forage maturity; digestion kinetics; *in sacco*; *in vitro*; dairy cows.

INTRODUCTION

Diet composition is affected by forage species and stage of growth. The effect of maturity on digestion and animal performance results mainly from changes in plant morphology and in cell wall components, which affects dry matter (DM) intake and digestibility (Van Soest, 1994).

Maturity is the most important factor affecting forage quality (Murphy, 1990). Forage quality is never static; plants continually change in quality as they mature. As plant cell-wall content increases, indigestible lignin accumulates, and, in late spring, grass maturity changes so rapidly that it is possible to measure significant declines in forage quality every two or three days (Cherney & Hall, 1997).

Forage maturity refers principally to the morphological development, especially the onset of certain stages of the reproductive cycle. As the plant matures, the chemical composition of the plant changes. A large part of this change is an increase in the proportion of plant stem relative to leaf; crude protein content decreases while structural carbohydrate and lignin contents increase. Along with these changes is a decrease in the leaf/stem ratio. The stems increase in height, and consequently the stem fraction makes up a greater portion of plant dry matter (Chaves *et al*., 2001).

Digestibility is a useful measure of pasture quality and refers to the proportion of a feed an animal can use to satisfy its nutritional requirements. Forage digestibility decreases as the plant matures, the indigestibility of grass stem components accounting for the greatest part of this decrease. In grasses, leaf digestibility does decrease, however, the majority of change results from changes in the proportion and digestibility of stems with maturity. The leaf component of legumes (e.g., white clover) does not change significantly with age.

As forage matures, the slow passage of digesta through the gut and the attendant poor fermentation of the refractory fibre, result in low voluntary feed consumption and poor animal performance (Kennedy & Murphy, 1988).

Accurate prediction of performance for animals fed ryegrass diets and options for supplementation with high-quality forages requires a greater understanding of how digestion processes are influenced by the stage of maturity.

Our objective was to analyze and compare changes in composition and digestion of DM and crude protein (CP) of ryegrass growing to maturity in spring.

METHODS

A one-year-old pure perennial ryegrass pasture (*Lolium perenne* L. cv. Grasslands Samson) was used for this study. A 15m by 25m plot was divided into three equal areas separated by trimmed pathways and mown on either 21/08/2000 (area one), 11/09/2000 (area two) or 21/09/2000 (area three). About 2 kg of forage was cut from each area at 7-14 day intervals for analysis. Cutting continued until mid December, but data presented here relate to relatively young forage, sampled on 11/09, 21/09, 13/10, 03/11 and 17/11/2000.

Samples were cut with electric clippers to approximately 5 cm above the soil level. From area one, ryegrass regrowth was harvested at 21, 31, 53 and 74 days; from area two ryegrass was harvested at 10, 32, 53 and 67 days and at 22, 43 and 57 days from area three. The areas harvested decreased as the ryegrass matured, and material was stored at -18°C until analysis. The schedule below indicates cutting dates and age (days) of the forage cut during the trial.

Area	**1**	**2**	**3**
		Initial harvest date	
Sample dates	21 August	11 September	21 September
11 September	21		
21 September	31	10	
13 October	53	32	22
3 November	74	53	43
17 November		67	57

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

[3] Statistics Research and Consulting Centre, Massey University, Private Bag 11222, Palmerston North, New Zealand

This design enables nutritive value of ryegrass to be monitored at different ages and harvest dates. The time of the year (harvest date) may affect rate of change in nutritive value.

Analysis

Grass samples were held frozen during preparation for analysis, including chopping into 2 cm lengths prior to being minced in a Kreft Compact meat mincer. The mincer had 12 mm diameter holes in the sieve plate, resulting in particle size distribution similar to that of chewed material (Barrell *et al*., 2000). Minced forage was used for *in sacco* and *in vitro* incubations using methods described by Burke *et al*. (2000) and Chaves *et al*. (2001) and for analysis of composition by NIRS (Corson *et al*., 1999) supported by wet chemistry analysis.

In sacco procedures

All incubations were carried out in one rumminally fistulated cow to avoid between-cow effects because the work focused on ryegrass degradation kinetics as affected by maturity and harvest date. Each incubation run evaluated three maturity/harvest date selections with ryegrass standard enabling comparison between cow incubations. Duplicate dacron bags (35 μm pore size) containing about 25-30 g wet weight were incubated for each stage of maturity for 2, 6, 12, 24 and 72 hours. Bags were placed in weighted lingerie bags to ensure a similar location within the rumen and to facilitate removal. Bags representing 0h were not placed in the rumen, and all bags were washed to remove soluble material prior to drying at 60°C for 24 hours and analysis (Chaves *et al*., 2001). After the appropriate incubation period, washing and drying, the bags were weighed and analysed to determine disappearance of DM, CP and other constituents.

Kinetics of digestion

Disappearance of DM and CP was analysed using a non-linear model (model no. 1) described by Lopez *et al*. (1999) in to determine fractional disappearance rate (k, %/hour) and potential degradation (P) according to:

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

Where A = soluble DM (% of DM washed out of bags at t = 0h), B = degradable insoluble DM, and t = time in hours.

Model parameters were estimated with the non-linear (NLIN) procedure of SAS (2001), which is appropriate for situations that do not have long digestion lag times. The model is able to reduce the residual deviations from the model equation for both degradation rate and A and B estimates (Nocek & English, 1986). Separate curves were calculated for each combination of initial harvest date and physiological age.

In vitro procedure

The *in vitro* incubations were carried out in conjunction with *in sacco* measurements using rumen inoculum from the same cow. About 2.5 g of minced pasture was incubated in 50 ml bottles with buffer and reducing agent under anaerobic conditions (Burke *et al*., 2000). Triplicate samples were removed after 0, 2, 4, 6, 8, 10, 12 and 24h of incubation for determination of ammonia concentrations to indicate proteolysis, and VFA concentration at 0, 6, 12 and 24h (Burke *et al*., 2000; Chaves *et al*., 2001).

Statistical analyses

Changes in the parameters with physiological age and initial harvest date were investigated by fitting a general linear model (GLM) to each parameter (e.g., A, B, k, E), with one factor: initial harvest date and several covariates (e.g., lignin concentration in original forage, physiological age, physiological age^2, initial harvest date x physiological age and initial harvest date x physiological age^2). This equation enabled both linear and quadratic relationship to be tested. Observations were weighted by 1/Standard Error^2, giving a higher weighting and more precise estimates.

RESULTS

Changes in nutritive value

Crude protein concentration in the DM declined rapidly with maturity (Table 1) from about 22.5% at 21 days to 10.0% after 57 days in area one, 18.7% at 10 days to 7.2% after 67 days in area two, and 14.7% at 22 days to 8.2% after 57 days in area three. These changes were associated with increases in the neutral detergent fibre (NDF) fraction of the DM (42.1% to 50.9%; 47.7% to 56.3% and 47.6% to 53.4% for area one, two and three respectively) as well as the acid detergent fibre (ADF) fraction of the DM (Table 1). The acid detergent lignin (ADL) percentage of DM increased by a small amount as the ryegrass matured from about 2.61% at 22 days to 2.83% at the final harvest (Table 1). Soluble carbohydrate concentration showed an increase with maturity.

The feeding value of mature ryegrass was also interpreted in relation to cow energy requirements for maintenance and milk production, by estimating the net energy for lactation (NE_L) and maintenance (NE_m). NE_L is an estimate of the amount of energy from grass incorporated into milk produced from a cow fed these diets and NE_m is an estimate of the amount of energy required for maintenance (NRC, 2001). As ryegrass matured, the NE_L (MJ/kg grass DM) decreased from 7.34 MJ/kg at 21 days of age to 6.38 MJ/kg at 74 days in area one. In contrast, NE_m was similar for all physiological ages (averaging 6.90, Table 1). Late-cut grass (area three) had consistently lower nutritive value than grasses cut earlier but at similar physiological ages since cutting. Grass from area three had the highest NDF and ADF concentrations reaching an average of 50.2% and 29.8% of the DM respectively, with 8.2% crude protein (CP) and 2.81% lignin (ADL) at day 53.

DM and CP kinetics

Table 2 summarises the kinetic parameters for DM and CP. The percentage DM in the soluble "A" fraction of grasses harvested from the three areas averaged 38% of DM and was not affected by initial harvest date, lignin concentration or physiological age. Fifty seven percent of the variation in the value for "A" (DM) was explained by initial harvest date, physiological age and lignin

TABLE 1: Chemical composition (g/100 g dry matter (DM)) and predicted nutritive value of ryegrass cut at different physiological ages (days) and initial harvest dates (area 1, 2 and 3).

Area	Age	H	DM	CP	CHO	NDF	ADF	OMD	ADL	NE_m [a]	NE_L [b]
1	21	18	19.1	22.5	6.9	42.1	23.5	78.3	2.66	7.03	7.34
1	31	19	19.2	17.2	6.6	48.0	26.1	78.1	2.38	7.10	6.69
1	53	32	19.5	14.6	5.2	48.6	29.3	76.2	2.50	6.87	6.63
1	74	48	22.9	10.0	9.9	50.9	30.0	75.4	2.62	6.95	6.38
2	10	11	18.2	18.7	5.0	47.7	27.8	74.8	2.68	6.66	6.73
2	32	26	17.8	16.8	4.6	45.6	28.3	77.8	2.66	6.96	6.96
2	53	42	23.4	10.4	9.7	50.3	29.9	75.4	2.36	6.94	6.44
2	67	58	28.9	7.2	9.8	56.3	30.9	72.9	3.07	6.73	5.78
3	22	21	19.1	14.7	5.4	47.6	29.2	76.7	2.49	6.92	6.73
3	43	35	23.0	11.1	10.3	49.6	28.9	76.1	2.28	7.04	6.52
3	57	47	26.6	8.2	10.0	53.4	31.4	72.9	2.81	6.70	6.10
Mean[c]	42	32	21.6	13.8	7.6	49.1	28.7	75.9	2.59	6.90	6.57

Abbreviations: Age, physiological age; H, pasture height (cm); CP, crude protein; CHO, soluble carbohydrates; NDF, neutral detergent fibre; ADF, acid detergent fibre; OMD, organic matter digestibility; ADL, acid detergent lignin; NE_m, net energy for maintenance (MJ/kg); NE_L, net energy for lactation (MJ/kg).

a – Prediction equation from NRC (2001).

b - Prediction equations from Mertens *et al.* (1993).

c – Mean for all physiological ages and areas.

concentration. In contrast to DM, the percentages of CP in the "A" fraction (average 48% of DM) was affected by both initial harvest date ($P<0.001$) and lignin concentration ($P<0.001$) but not by physiological age, and the model explained 98% of the variation in "A" for CP.

The percentage of slowly degradable DM in the "B" fraction of grasses harvested from area one, two and three averaged 57, 57 and 58 respectively (Table 2) and was not affected by either initial harvest date or lignin concentration but this fraction was affected by physiological age ($P<0.01$). The model explained 80% of the variation with initial harvest date, physiological age and lignin concentration for "B" value for DM ($r^2=0.80$). The slowly degradable "B" fraction of CP had similar values as the "A" parameter (48% of total CP) and was affected by initial harvest date, lignin concentration and physiological age ($P<0.05$). The model explained 99% of the variation among all the parameters for "B" in CP disappearance.

The fractional disappearance rate for DM (k; 0.053) of grasses harvested from all areas was not affected by initial harvest date but was affected by both lignin concentration and physiological age ($P<0.05$), which accounted for 72% of the variation in k. In contrast to the k value for DM, the k value for CP averaged 0.088/h and was not effected by physiological age but 89% of variance was explained by affected by initial harvest date and lignin concentration ($P<0.05$), so the model was better able to explain rates of degradation for protein than DM.

The effective degradability (E) which takes into account the effect of passage from the rumen and was calculated using an assumed fractional passage of 0.06 h^{-1}, averaged 67, 63 and 64 for grasses harvested from areas one, two and three respectively, and was affected by both physiological age and by initial harvest date ($P<0.05$) but was only marginally affected by lignin concentration ($P=0.10$). The model explained 81% of the variation in DM degradability based on initial harvest date, physiological age and lignin concentration. The effective degradability for CP averaged 76 but was not affected by initial harvest date, lignin concentration or physiological age.

In sacco

In sacco dry matter disappearance data is illustrated in Figure 1 and is summarised in Table 2. Figures 1A, B

TABLE 2: Ryegrass dry matter (DM) and crude protein (CP) degradation characteristics (% of DM) defined as soluble DM or CP (A), degradable insoluble DM or CP (B), fractional degradation rate (k, h^{-1}) and effective degradability (E) [a].

Area [b]	**Physiological Age**	**DM**				**CP**			
	(days)	**A**	**B**	**k**	**E**	**A**	**B**	**k**	**E**
1	21	40	58	0.067	71	49	51	0.091	80
1	31	38	60	0.058	67	46	54	0.075	76
1	53	35	59	0.071	67	45	51	0.126	79
1	74	42	52	0.044	64	46	46	0.095	74
2	10	39	60	0.044	65	50	50	0.065	76
2	32	37	59	0.050	64	41	56	0.101	76
2	53	39	51	0.058	65	41	51	0.117	75
2	67	37	59	0.035	59	62	34	0.036	75
3	22	30	65	0.067	65	34	61	0.122	75
3	43	40	54	0.049	64	45	48	0.088	73
3	57	42	56	0.038	64	65	31	0.052	79
Mean [c]	42	38	58	0.053	65	48	48	0.088	76

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

b - The three area represent initial harvest dates of 21/08 (area 1), 11/09 (area 2) and 21/09/2000 (area 3).

c- Mean for all physiological ages and areas.

and C show similar DM degradation rates for mature ryegrass from each area although young ryegrass has faster degradation rates than mature ryegrass (P<0.05). Differences in DM disappearance for grass harvested at different ages were most apparent at the 12 and 24h incubation times, which would affect rumen clearance. Total digestion (after 72h of incubation) was similar for all materials tested (averaging 91.7% disappearance).

In vitro

Products of degradation are indicated (Figure 1) by net ammonia production from area one, two and three (Figure 1D, E and F, respectively), and suggest there is insufficient N for microbial growth in grass older than 31 days because net ammonia concentration is observed to decline below the initial (time 0h) values during the incubation periods. The deficit in CP for microbial growth is indicated by concentration in the *in vitro* bottles, but net production excludes plant nitrogen incorporated into bacterial protein (Barrell *et al*., 2000). Ammonia concentration was affected by both physiological age and time of incubation (P<0.001) but was not affected by initial harvest date.

Maximum rates of total volatile fatty acids production occurred during the first 6 hours of the *in vitro* incubation (Table 3). Between 6 and 12 hours net VFA production did not show consistent patterns and the lowest production rate occurred between 12 and 24 hours of incubation (P<0.001). Total VFA production was not affected by either physiological age or initial harvest date but differed in concentration during incubation (P<0.001).

There were no statistical differences in proportions of VFA associated with physiological age, initial harvest date or incubation time (Table 3).

DISCUSSION

The primary purpose of this study was to establish the limits of digestion imposed by ryegrass maturation. Data presented here confirm the slow degradation of mature ryegrass, but also suggests a relatively lower nutritive value of ryegrass harvested later with the same physiological age (Table 1), despite high concentrations of soluble carbohydrates.

In contrast, different harvesting dates did not produce different values for the fractions of A, B or k and only show differences in the estimated DM degradation (E; P<0.05). Burke *et al*. (2000) measured a fractional degradation rate for immature ryegrass (k=0.114) that was higher than that for all physiological ages in this work. That difference in fractional degradation rate can be most likely explained by the cell wall of ryegrass epidermis becoming thickened, lignified, and completely covered with a cuticle and waxy layer with age, and this development is usually more pronounced for stem than leaf (Wilson, 1990; Chaves *et al*., 2001). It is well known that lignin reduces the degradation of plant material by rumen microbes. Lignin concentration, expressed in % of DM (%ADL), ranged from 2.5% to 3.1% (Table 1) and showed strong interaction with the decrease in the rate of degradation of DM and CP (P<0.05).

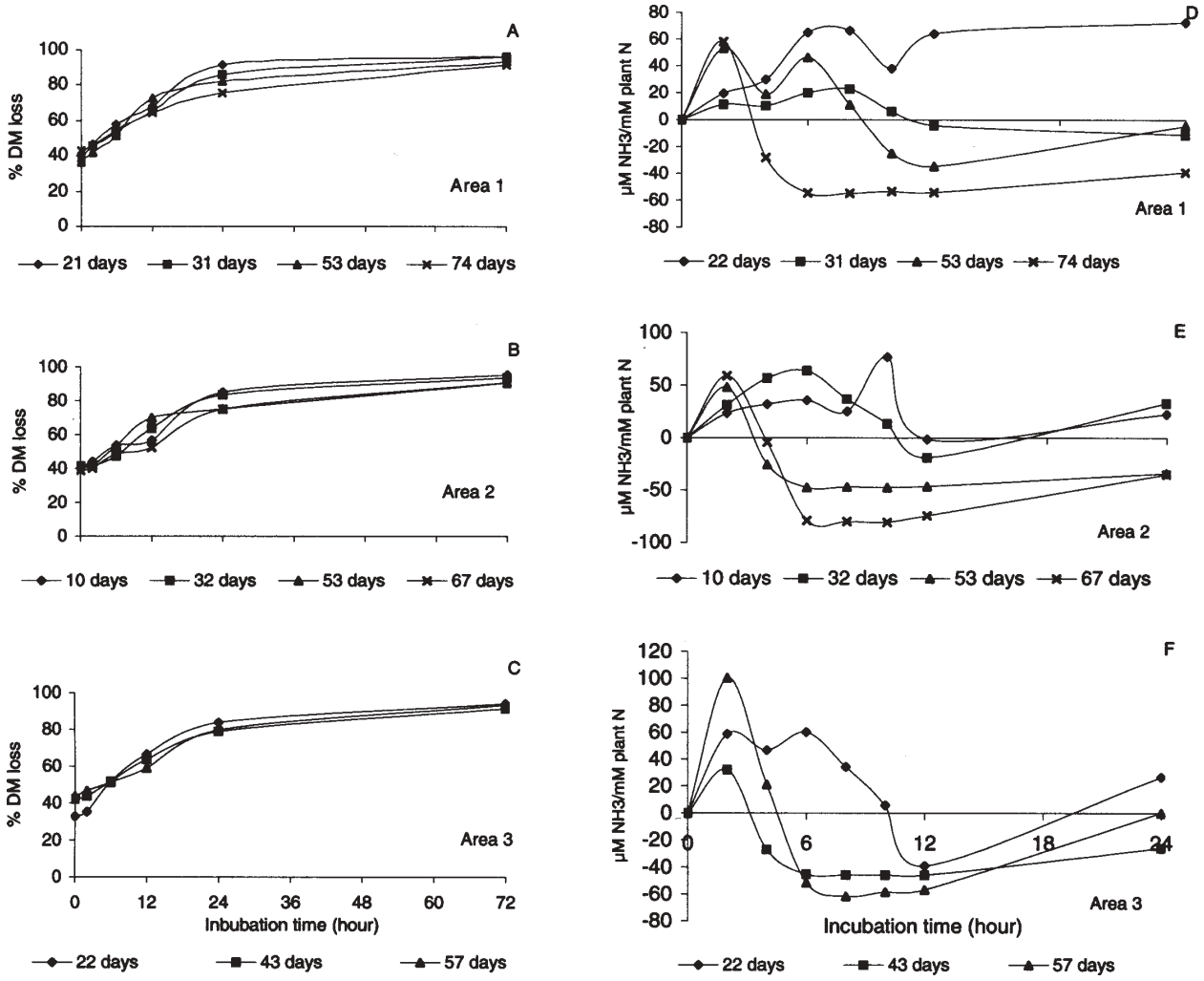
The rate and extent of protein degradation in the rumen is crucial, as it determines the availability of nitrogen to microorganisms and amino acids for absorption from the small intestine. Although lignin concentration did not appear to have any effect on the soluble DM, or degradable insoluble DM, and only a slight effect on effective degradability (P=0.10), lignin concentration did show an effect on the parameters A, B and k for CP (P<0.05). The protein consumed by the animal should be partly degradable in the rumen; peptides and amino acids derived from proteolysis stimulate microbial growth and rumen fermentation. It is, therefore, very important to determine the CP degradability (Table 2) and ammonia production (Figure 1) for ryegrass in different stage of maturity. Rapid degradation of dietary protein from ryegrass leads to decreased protein efficiency and contributes to an excessive formation of ammonia. As a consequence, excessive CP degradation may be the most limiting nutritional factor in high-quality temperate forages (Broderick, 1995). The analysis of variance for CP disappearance did not show significant differences between physiological ages. However, in area three, younger ryegrass with a high protein content (Table 1) had higher protein degradation rate than older ryegrass (Table 2); and both lignin concentration and initial harvest

TABLE 3: Total volatile fatty acid (VFA) production (mM/g dry matter/hour) and acetate: propionate ratio from *in vitro* incubations during the intervals 0 to 6, 6 to 12 and 12 to 24 hours of ryegrass in different stages of maturity.

Area	Physiological age (days)	Yield of total VFA (mM/g DM / h)			Acetate: propionate ratio		
		0 - 6 h	6 – 12 h	12 – 24 h	0 - 6 h	6 – 12 h	12 – 24 h
1	21	0.34	0.34	-0.04	2.89	3.29	5.87
1	31	0.34	0.10	0.14	2.30	2.20	3.22
1	53	0.19	0.26	0.05	3.18	3.41	3.99
1	74	0.30	0.19	0.03	2.51	1.98	10.51
2	10	0.29	0.12	0.11	2.27	3.32	2.67
2	32	0.23	0.22	0.11	3.92	4.00	4.18
2	53	0.27	0.20	0.06	2.26	2.23	5.95
2	67	0.55	0.14	0.07	3.62	3.57	3.77
3	22	0.27	0.15	0.08	3.26	3.27	2.73
3	43	0.28	0.13	0.07	2.14	1.62	3.14
3	57	0.50	0.18	0.04	2.84	9.70	2.40
Mean [a]	42	0.32	0.18	0.07	2.84	3.51	4.40

a - Mean for all physiological ages and areas.

FIGURE 1. *In sacco* dry matter disappearance (A), (B) and (C) and *in vitro* ammonia production (D), (E) and (F) from ryegrass sampled at 3 or 4 physiological ages and from area 1, 2 and 3. See text for details.



date affected CP degradation ($P<0.05$). Chesson (1988) showed that lignin had a much greater influence on nutrient availability than would be expected based on its concentration in the feed. Lignin is considered the overriding factor in determining the availability of structural polysaccharides and protects the cell wall as a whole, rather than selected components, from microbial attack (Wilson, 1993).

This work confirms data from Kennedy & Murphy (1988), showing that, as forage matures, the slow passage of digesta through the gut and the attendant poor fermentation of the refractory fibre will result in low voluntary feed consumption and poor animal performance. The resistance of lignocellulose to particle-size reduction is an important property, because digesta must be made small enough to pass from the rumen and alleviate the inhibition by distension of voluntary feed intake. Also, reduction of digesta particle size by chewing and rumination increases the surface area exposed to microbial attack, and digestion increases the functional specific gravity of the particles, which enhances their probability of passage from the rumen (Akin, 1979).

The supplementation of grass with different forages (FMR, forage mixed ration) to optimize milk yield is challenging because of varying forage quality and the difficulty in quantifying intake. The performance of grazing cows differs significantly from cows fed total mixed ration in dry matter intake, milk production, milk protein content, live weight, and body condition score (Kolver & Muller, 1998). However, the rapid and extensive degradation of the CP fractions in ryegrass may limit the supply of metabolizable amino acids that is necessary for milk synthesis (Table 2 and Figure 1D, E and F). Milk protein synthesis in dairy cows that consume diets containing large amounts of rumen degradable protein (RDP), such as New Zealand pastures, can be increased by the supplementation of fermentable carbohydrates, which optimize ruminal microbial protein synthesis, or by the supplementation of ruminally undegradable protein (RUP) that is available in the small intestine (Nocek & Russell, 1988). Ideally, carbohydrates and protein should be supplied in a ratio that optimizes microbial protein synthesis and flow of microbial N to the small intestine (Carruthers *et al*., 1997). Future studies will use a dairy nutrition model (CNCPS) to develop strategies for high milk production within a grazing system and predictions will be evaluated against data from animal feeding trials.

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REFERENCES

Akin, D.E. 1979: Microscopic evaluation of forage digestion by rumen microorganisms-a review. *Journal of animal science 48*: 701-710.

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

Broderick, G.A. 1995: Desirable characteristics of forage legumes for improving protein utilization in ruminants. *Journal of animal science 73*: 2760-2773.

Burke, J.L.; Waghorn; G.C.; Brookes, I.M.; Attwood, G.T. 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.

Carruthers, V.R.; Neil, P.G.; Dalley, D.E. 1997: Effect of altering the non-structural: structural carbohydrate ratio in a pasture diet on milk production and ruminal metabolites in cow in early lactation. *Animal science 64*: 393-402.

Chaves, A.V.; Waghorn, G.C.; Brookes, I.; Burke, J. 2001: Digestion kinetics of mature grasses. *Proceedings of the New Zealand Society of Animal Production 61*: 8-12.

Cherney, J.H.; Hall, M.H. 1997: Putting forage quality in perspective. http://www.cas.psu.edu/docs/casdept/agronomy/forage/forages.html

Chesson, A. 1988: Lignin-polysaccharide complexes of the plant cell wall and their effect on microbial degradation in the rumen. *Animal feed science and technology 21*: 219-228.

Corson, D.C.; Waghorn, G.C.; Ulyatt, M.J.; Lee, J. 1999: NIRS: Forage analysis and livestock feeding. *Proceedings of the New Zealand Grasslands Association 61*: 127-132.

Kennedy, P.M.; Murphy, M.R. 1988: The nutritional implications of differential passage of particles through the ruminant alimentary tract. *Nutrition research reviews 1*: 189-208.

Kolver, E.S.; Muller, L.D. 1998: Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. *Journal of dairy science 81*: 1403-1411.

Kolver, E. S.; Muller, L.D.; Barry, M.C.; Penno, J.W. 1998: Evaluation and application of the Cornell Net Carbohydrate and Protein System for dairy cows fed diets based on pasture. *Journal of dairy science 81*: 2029-2039.

Lopez, S.; France, J.; Dhanoa, M.S.; Mould, F.; Dijkstra, J. 1999: Comparison of mathematical models to describe disappearance curves obtained using the polyester bag technique for incubating feeds in the rumen. *Journal of animal science 77*: 1875-1888.

Mertens, D.R.; Undersander, D.; Thiex, N. 1993: Association **recommends** forage analyses procedures. http://www.foragetesting.org/fap/appendixa.html

Murphy, M.R. 1990: Chemical and physical properties of lignocellulose that limit intake and digestion. *In*: Akin, D.E.; Ljungdahl, L.G.; Wilson, J.R.; Harris, P.J. *ed*. Microbial and plant opportunities to improve lignocellulose utilization by ruminants. Proceedings of the tri-national workshop microbial and plant opportunities to improve lignocellulose utilization by ruminants. p. 17-22.

National Research Council, 2001: *Nutrient requirement of dairy cattle*, 7th revised edition. National Academy Press, Washington, DC.

Nocek, J.E.; English, J.E. 1986: In situ degradation kinetics: evaluation of rate determination procedure. *Journal of dairy science 69*: 77-87.

Nocek, J.E.; Russell, J.B. 1988: Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *Journal of dairy science 71*: 2070-2107.

SAS. 2001: The SAS system for Windows version 8.02, SAS Institute, Cary, NC, USA.

Van Soest, P.J. 1994: Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, NY.

Wilson, J.R. 1990: Influence of plant anatomy on digestion and fibre breakdown. *In*: Akin, D.E.; Ljungdahl, L.G.; Wilson, J.R.; Harris, P.J. *ed*. Microbial and plant opportunities to improve lignocellulose utilization by ruminants. Proceedings of the tri-national workshop microbial and plant opportunities to improve lignocellulose utilization by ruminants. pp 99-117.

Wilson, J.R. 1993: Organization of forage plant tissue. *In*: Jung, H.G.; Buxton, D.R.; Hatfield, R.D.; Ralph, J. *ed*. Forage cell wall structure and digestibility. Based on the international symposium on forage cell wall structure and digestibility. USDA-Agricultural Research Service and the U.S. Dairy Forage Research Center, Madison, Wisconsin, 7-10 Oct. 1991. pp 1-32.