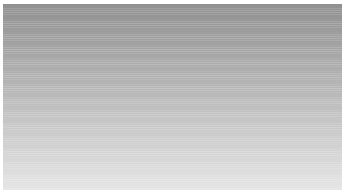

End of Project Report

Project No. 3960

OPTIMISATION OF NUTRIENT SUPPLY FOR BEEF CATTLE FED GRASS OR SILAGE

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Teagasc acknowledges with gratitude the support of European Union Structural Funds (EAGGF) in financing this research project.

Beef Production Series No. 26

GRANGE RESEARCH CENTRE, Dunsany, Co. Meath

ISBN 1 8 4170 169 6

May 2001

Teagasc
Agriculture and Food Development Authority

EUROPEAN UNION

European Agricultural Guidance and Guarantee Fund

SUMMARY AND CONCLUSIONS

Since forage forms a large part of growing ruminant rations in Ireland, the trust of this project was to examine the effect of ensilage on ruminal digestion of grass and to examine ruminal microbial protein and intestinally absorbable protein supplied by grass and/or clover. A range of *in vitro* and *in vivo* techniques were employed and strategies used by commercial beef producers to optimise cattle growth (and nutrient supply) were also documented.

To accomplish the aims of this project, a range of methodology developments/modifications *in vitro* and *in vivo* was carried out. From *in vitro* methodology development it was concluded that :

(i) Compared with fresh silage, drying *per se* may give artifically higher rates of dry matter (DM) digestion.

(ii) Greater experimental precision can be obtained by ensuring a greater substrate surface area to reaction volume ratio in each reaction vessel.

(iii) For studies where the rate of digestion is of greatest importance, pre-incubation of frozen inoculum in a nutrient medium best simulated the cellulolytic activity of unfrozen inoculum. In studies that require large volumes of inoculum for extended work, freezing directly is justified.

(iv) Neutral detergent extraction altered *in vitro* digestion characteristics of silage. The residue after washing with water at 70°C has a high residual fibre concentration and is more representative of the structural components of silage ingested by ruminants.

(v) A semi-continuous culture system developed at Grange Research Centre can successfully model *in vitro* ruminal digestion of fibre and starch-based diets in a controlled environment.

From *in vivo* methodology development it was concluded that :

(i) Oven drying at 60°C and correction for loss of volatiles gives a good estimation of DM concentration of ruminal particulate digesta. This procedure has the added advantage that drying at 60°C allows the residual materials to be analysed for fibre fractions without concern for heat damage which can occur at a higher drying temperature.

(ii) A naso-ruminal sampling device can be used to measure the relative patterns of fermentation of contrasting diet types when *in situ* for up to 7 days.

(ii) Application of a vacuum to withdraw samples had no negative effect on ruminal fluid variables.

From *in vitro* studies on grass digestion, it was concluded that :

(i) Ensiling of grass decreased the apparent extent of digestion of cell walls when in the presence of the whole plant and that this largely reflected an increase in the lag time before digestion commenced.

(ii) Ensiling of grass did not negatively affect the digestion of isolated cell walls.

(iii) There is a negative impact of ensiling on microbial protein production from the water soluble carbohydrate fraction of grass.

(iv) Supplementation with the water soluble fraction of grass significantly improved the apparent extent of digestion for ensiled forages when compared with the supplementation of the post-ensiling fraction in a batch culture system.

(v) There is a negative impact of maturity on the pattern of cell wall fermentation and that this impact can be decreased by ensiling method.

From studies on herbage digestion *in vivo* it was concluded that :

(i) Grass silage type had a greater effect than the rate of concentrate fermentation on ruminal microbal protein synthesis.

(ii) Harvesting time had a bigger impact on nutrient supply from herbage than sward type (grass or grass/clover).

(iii) Increasing clover content in the herbage decreased the biological value (g nitrogen retained/kg absorbed) of dietary protein.

Diverse stratgies were used on commercial beef farms to optimise nutrient supply and animal growth. Average animal performance on individual farms was not better than would be typically recorded in a research environment. There was scope on many of the farms to improve technical performance and to decrease the costs of production.

INTRODUCTION

Optimisation of microbial growth and activity in the rumen requires the simultaneous presence of appropriate forms of nitrogen and energy. Because of the range of ingredients of varying composition available for inclusion in ruminant rations, it is difficult to empirically predict and optimise the pattern of nutrient supply from the rumen. To put ration formulation on a more mechanistic basis, information is needed on the digestion patterns of substrates, (proteins, simple and complex carbohydrates, lipids) supplied by ingredients rather than just the intact ingredients themselves. This information is difficult to obtain *in vivo* and consequently, diverse *in vitro* techniques have been developed to address specific questions with a degree of control generally not possible *in vivo*. Such techniques however, are part of a battery of approaches to understanding ruminal digestion and serve to complement rather then to replace *in vivo* studies.

Since forage forms a large part of growing ruminant rations in Ireland, the trust of this project was to examine the effect of ensilage on ruminal digestion of grass and to examine microbial protein and intestinally absorbable protein supplied by grass and/or clover. A range of *in vitro* and *in vivo* techniques were employed and strategies used by commercial beef producers to optimise cattle growth (and nutrient supply) were also documented.

The overall ojectives of this project were (i) to advance the understanding of digestion of silage in the rumen, (ii) to provide preliminary information on nutrient supply from grazed herbage, (iii) to document ration formulation strategies in practise and (iv) to develop the methodology required to address the above objectives.

METHODOLOGY DEVELOPMENT

To accomplish the aims of this project, a range of methodology developments/modifications *in vitro* and *in vivo* was carried out

In vitro

Experiment 1 :

A comparison of sample preparation techniques for studies on *in vitro* digestion of silage by ruminal microorganisms

In many *in vitro* studies, feedstuffs are dried and milled for convenience of handling and sample homogeneity. Such preparation procedures can alter biochemical composition and subsequent digestion of the feedstuffs. The aim of this experiment was to identify the optimal sample preparation technique for *in vitro* studies with grass silage.

Ensiling conditions were manipulated to give a restricted-well-preserved, an extensively fermented well-preserved, and a poorly-preserved grass silage. Dry matter (DM) digestibility (DMD), ammonia-N, lactic acid and volatile fatty acid (VFA) concentrations for the three silages were, respectively, 589, 589 and 532 g/kg; 3.8, 8.5 and 10.8 g/kg DM, 48.7, 85.2 and 4.0 g/kg DM and 16.9, 99.4 and 37.1 g/kg DM. Sample preparation techniques compared were fresh (chopped to 1 cm), frozen (chopped to 1 cm), oven dried at 40°C for 48 h (milled to 2 mm), freeze dried (chopped to 1 cm) and freeze dried (milled to 2 mm). Samples were incubated *in vitro* for 0, 3, 6, 12, 18, 24, 48, 72 h, in buffered rumen fluid and the kinetics of DM digestion were recorded. No significant differences were observed in the kinetics of digestion of a standard silage between *in vitro* incubations indicating consistency in microbial activity. There was no main effect of silage type on the kinetics of DM digestion. The average rate (per hour) and extent of digestion (g/kg initial DM) for the fresh, frozen, oven-dried, freeze-dried (1cm) and freeze-dried (2 mm) treatments

were 0.0164, 0.0159, 0.037, 0.0335 and 0.0288 (s.e.d. 0.00711) and 29.2, 35.7, 37.6, 30.6 and 32.2 (s.e.d. 3.16), respectively. It is concluded that, compared with fresh silage, drying *per se* may give artificially higher rates of DM disappearance *in vitro*.

Experiment 2 :

Sources of variation *in vitro* digestion of silage by ruminal microorganisms

Variation within *in vitro* studies can be attributed to "real" differences between feedstuffs and "imposed" differences arising from the technique used. The aim of this experiment was to identify and minimise "imposed differences" that may arise in the modified Tilley and Terry technique. Two potential sources of "imposed" variation were examined. In experiment 2a, the influence of substrate surface area: inoculum ratio (SA:V) in fermentation units on the variance within replicates at different times of incubation of grass silage was examined. Residual neutral detergent fibre (NDF) was quantified at the end of incubation. The SA:V ratio of the fermentation units was altered by a modified design and reorientation of the units in an incubator giving a 1:2 ratio with vertical agitation (T1) and a 1:1.6 ratio with horizontal agitation (T2). Variance within replicates was significantly reduced with T2 ($p<0.05$). It is concluded that greater experimental precision can be obtained by ensuring greater SA:V ratios.

In experiment 2b, inoculum preservation to eliminate variation that occurs with the repeated collection of rumen fluid from silage-fed donor animals was examined. The effect of freezing directly (T2), freezing with and without the presence of a cryoprotectant (T4 and T3, respectively) and the impact of an incubation step preinoculation on T3 and T4 (T5 and T6, respectively) on the resultant cellulolytic activity of the inoculum were compared with an unfrozen inoculum (T1). A beneficial effect of pre-incubation on both lag (h, (se 1.43)) and extent (g, (s.e. 0.013)) parameters was found (21.52 and 0.348, 34.12 and 0.359, 12.4 and 0.387, 10.21 and 0.429 for T3, T4, T5 and T6

respectively) when compared with those for T1 (control) (0.41 and 0.432). In all cases there was a significant lag in the pattern of NDF disappearance but the fractional rate constant (/h, (se 0.022)) was not affected by method of preservation (0.07, 0.102, 0.103, 0.094, 0.099 and 0.069 for T1, T2 T3 T4 T5 and T6, respectively). It is concluded that, for studies where the rate factor is of greatest importance, T6 best simulated the cellulolytic activity of T1. In studies that require large volumes of inoculum, for extended work, freezing directly is justified.

Experiment 3 :

The effect of aqueous or detergent extraction on the *in vitro* digestion of the cell wall fraction of grass silage

The separation of forages into soluble and insoluble nutrient fractions is often necessary as these fractions can differ in their digestion kinetics and end products of their fermentation. The residue remaining after neutral detergent (ND) extraction is considered to represent the cell wall fraction of forages. However the severity of ND for the isolation of cell walls, may influence subsequent *in vitro* digestion kinetics. The aim of this experiment was to examine the effect of extraction medium (water and ND solution) on the chemical composition and *in vitro* digestion kinetics of the cell wall fraction of perennial ryegrass silage.

A perennial ryegrass silage was dried at 40°C for 48 h and the dried material chopped to 1 cm (DM). Forages were washed with cold water for 30 min, submersed in 8 l of water and with continuous agitation, the temperature of the water was raised and maintained at 70°C for 1 h. The cold wash was repeated and the residue dried at 40°C for 48 h (F70). The NDF of DM was extracted according to standard procedures. Each fraction was incubated with rumen fluid and fermentation units were sampled in triplicate 7 times over a 72-h period. Residues were recovered by vacuum filtration through 100µm mesh and washing with water (20°C). Recovered residues

were dried at 40°C for 48 h, weighed and the ND residue measured.

The concentration of neutral and acid detergent fibre(ADF) was increased with extraction, and the respective concentrations were lower in the F70 fraction (Table 1). Following extraction, the crude protein concentration and DMD decreased, with the effect on the latter more severe for ND extraction. The rate of degradation was not affected by extraction procedure, relative to the unfractionated material but the lag was increased and the extent of digestion decreased by ND isolation when compared with the un-fractionated material and F70 fraction (Table 1), suggesting alterations in the structural component.

It is concluded that ND extraction altered the *in vitro* digestion characteristics of the forage in this study. Therefore the F70 fraction, which has a high residual NDF concentration is more representative of the structural component ingested by silage-fed ruminants.

Experiment 4 :

Development of a rumen simulation semi-continuous culture (RSSC)

In vitro continuous culture systems are designed to simulate rumen function, by combining the dynamics of *in vivo* systems with the control of *in vitro* systems. The purpose of this work was to finalise the development of a semi-continuous culture (by imposing pH control) and to examine the ability of the system to simulate *in vivo* fermentation of both starch-and fibre-based diets.

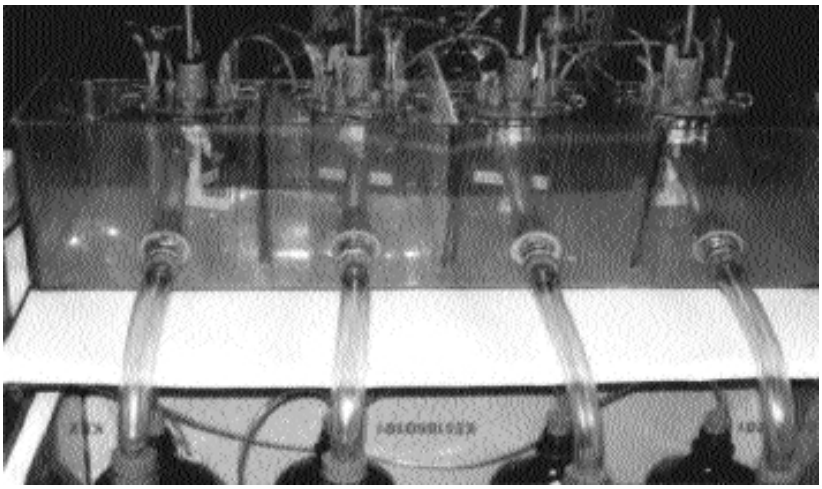
The RSSC is a semi-continuous dual flow culture system, which concurrently runs 4 fermentation vessels (Figure 1).

Table 1 . Chemical composition of silage components and kinetic parameters of *in vitro* ruminal digestion

	Component				
	DM	F70	NDF	Significance	sed
Dry matter (DM) digestibility	754.0[a]	643.0[b]	480.0[c]	***	28.3
Crude protein (g/kg DM)	162.0[a]	82.5[c]	97.93[b]	***	0.674
Neutral detergent fibre (g/kg DM)	504.5[c]	864.0[b]	885.0[a]	***	3.02
Acid detergent fibre (g/kg DM)	298.0[c]	505.7[b]	514.3[a]	***	4.44
Ash (g/kg DM)	82.1[a]	33.7[b]	37.2[b]	**	10.21
Lag (h)	10.0[a]	10.3[a]	38.2[b]	*	1.79
Rate (/h)	0.10	0.06	0.07	NS	0.013
Extent (g/100g incubated)	74.2	78.6	59.2	*	3.49

[a]Means with different subscripts differ significantly ($p<0.05$)

Figure 1. Rumen Simulation Semi-Continuous Culture



These can be maintained under anaerobic, constant temperature and homogenous mixing conditions. Flow dynamics within the system are based on *in vivo* studies. Controlled buffer infusion determines the liquid dilution rate (LDR), while the continuous removal of filtered fluid from the vessel interior determines the rate of solid flow from the vessel (solid dilution rate, SDR). Values are set at 6 and 3% h^{-1}, respectively.

The experimental protocol consists of a 10-day fermentation period. On Day 1, each vessel (working volume 1800 ml) is inoculated with rumen microorganisms collected from fistulated steers. The feeding rate is 45 g DM/vessel/day in two equal feeds every 12 hours. Each morning, filtered effluent, displaced effluent and saliva input volumes are sampled and recorded. Days 1-5 allow for the system to develop a "steady-state" environment. Sampling occurs on Days 6-10. The effectiveness of a teflon junction pH probe to maintain environmental pH between pH 6.2-6.8, over a 10 day period, in one fermentation vessel (VI), was examined. The probe was supported by a pH meter and peristaltic pump, which immediately infused 3M HC1 or 5M NaOH, when the recorded pH fluctuated outside the defined limits.

A second vessel (V2) was maintained under the same operating conditions without pH control. A fermentable fibre-based concentrate was used in each vessel (Diet F). The remaining two vessels (V3 + V4) were maintained under the same operation conditions without pH control but fed a starch-based concentrate (Diet S). Volatile fatty acid production and DMD were examined in all vessels. Results were compared with those obtained from an *in vivo* study, where ruminally-fistulated animals were fed a basal hay diet at 8 am daily, with *ad libitum* access to Diet F or Diet S.

The pH was successfully maintained between pH 6.2 and pH 6.8 during the steady-state period. This differed from the *in vivo* situation and may be attributed to different feeding systems. The non-glucogenic ratio [(acetate + butyrate)/propionate] did not differ significantly from the *in vivo* results for either diet (Diet F - pH control 5.2, Diet F - no pH control 4.6, Diet F - *in vivo* 5.2, Diet S - *in vitro* 1.4, Diet S - *in vivo* 1.5).

It is concluded that the rumen semi-continuous simulation culture developed at Grange Research Centre can successfully model *in vivo* rumen digestion of fibre and starch based-diets in a controlled environment.

In vivo

Experiment 5 :

The estimation of the DM concentration of ruminal particulate digesta

Investigation of ruminal digestion frequently requires estimation of the size of the pools of various fermentation products. For example, accurate estimates of the volume of liquid in the rumen require manual evacuation of rumen digesta and measurement of its DM concentration. Since the end-products of rumen digestion are volatile, the DM of a sample of ruminal digesta is likely to be underestimated by oven drying. For other fermented materials, measurement of DM concentration by toluene distillation with a correction of volatiles in the distillate is considered the most appropriate method available at present. Because of the hazardous nature of toluene, this is not an attractive routine procedure and fermented materials are frequently oven dried. The objective of this study was to examine the effect of oven-drying on the DM concentration of ruminal particulate digesta and to construct a relationship to allow correction of DM concentrations for loss of volatiles when drying.

Six ruminally-fistulated steers were offered one of three grass silages (no additive, formic acid or an inoculant) and 0 or 3 kg concentrates in a 6 (animals) by 6 periods) Latin square experiment. In one period of this experiment, the rumen of each animal was manually evacuated on three occasions over a 7 day period. The times of evacuation varied in relation to feeding to represent digesta pools 2, 6 and 24 h thereafter. A sample of particulate digesta was divided into 4 sub-samples. One sub-sample was dried to a constant weight at 60°C and another was dried at 98°C. The DM concentrations were recorded and volatile components were extracted into distilled water. The fluid phase of a third sub-sample was expressed and the concentrations of volatiles analysed therein. The DM concentration of a fourth sub-sample was measured by corrected toluene distilla-

tion. The sample set used had a mean (s.d.). DM (toluene) concentration of 127 (11.3) g/kg. The mean concentrations (g/kg DM) of ammonia, lactic acid (LA) and total volatile fatty acids (VFA) were 2.30 (1.32), 5.66 (1.22) and 57 (11.0), respectively. From the loss of volatiles on drying, the following correction equation was constructed: DM(60°C-corrected) = 1000 g DM (60°C) + 0.918 ammonia (g/kg DM) + 0.533 LA (g/kg DM + 0.86 VFA (g/kg DM). Digesta DM concentration measured by toluene distillation, oven drying at 60°C and oven drying at 60°C with correction was 127, 119 and 126 (s.e.d. 1.81), respectively.

It is concluded that oven drying at 60°C and correction for loss of volatiles gives a good estimation of DM concentration of ruminal particulate digesta. This procedure has the added advantage that drying at 60°C allows the residual material to be analysed for fibre fractions without concern for heat damage which can occur at a higher drying temperature.

Experiment 6 :

Comparison of procedures for the collection of ruminal fluid from cattle

Investigation of ruminal digestion often requires sampling of ruminal fluid. Ruminal fluid samples may be collected via a ruminal cannula or by stomach tube. Repeated sampling is not readily carried out by the latter procedure and samples may be contaminated by saliva produced during tube insertion and sampling. The first objective of this study was to evaluate the performance of a naso-ruminal sampling device which can remain *in situ* and so be used repeatedly without contamination. Ruminal fluid samples are collected in this laboratory from ruminally-fistulated animals by means of a vacuum pump. There is evidence that excessive suction force may increase ruminal fluid pH. The second objective of this study was to compare ruminal fluid samples aspirated using a vacuum pump or by the application of a mild vacuum using a syringe.

Four steers (bodyweight = 731 SD 64.1 kg) were used in each of two periods of 30 days duration. In period one, animals were offered 0.75 kg hay and 10 kg pelleted concentrates/head daily. The hay was offered at 0830 h and the concentrates in 2 equal meals at 0830 h and 1430 h. In period 2, the animals were offered 30 kg grass silage daily in two equal meals at 0830 hand 1430 h. On day 23 of each period, a naso-ruminal sampling device was inserted in each animal. On days 24 and 30, ruminal fluid samples were simultaneously collected via the naso-ruminal device using a 60 ml syringe or from four positions in the rumen using a vacuum pump at 0830, 1030, 1430, 1630 and 2030 h. pH was measured immediately and samples were preserved with 9 M sulphuric acid for chemical analysis. On day 34, ruminal fluid was also collected from 4 sites in the rumen using a 60 ml syringe.

In the comparison of the naso-ruminal and cannula methods, there were no effects of day of sampling or any day x method or sampling time (hours) x method interactions. The main effects of diet and method are summarised in Table 2. Individual VFA (expressed as a proportion of total VFA) did not differ between methods but the concentration of VFA in samples collected by the naso-ruminal sampler was 0.85 that of samples collected via the cannula. There was no significant difference between samples collected by syringe or by vacuum pump (Table 3).

It is concluded (i) that the naso-ruminal sampling device can be used to measure the relative patterns of fermentation of contrasting diet types when *in situ* for up to 7 days, and (ii) application of a vacuum to withdraw samples had no negative effect on ruminal fluid variables.

Table 2 . pH and concentration of metabolites in ruminal fluid collected via a naso- ruminal device(NASO) or a ruminal cannula (CANN)[1]

	Diet							
	Concentrate		Grass silage			Significance[4]		
	NASO	CANN	NASO	CANN	sed	M	D	MxD
pH	6.36	6.11	7.02	6.88	0.136	0.08	***	NS
Acetic acid[2]	48.2	55.7	32.9	42.2	2.32	***	***	NS
Propionic acid[2]	22.0	25.7	13.3	15.9	3.35	NS	**	NS
Butyric acid[2]	9.5	11.7	5.3	6.5	0.91	*	***	NS
Volatile fatty acids[2]	84.7	99.5	53.3	67.0	6.87	*	***	NS
Acetate : propionate	2.41	2.40	2.76	3.00	0.238	NS	*	NS
Ammonia[3]	100.1	102.7	79.2	88.3	21.45	NS	NS	NS

[1]Mean of samples collected at 5 times on 2 days for each of 4 animals/diet, [2]mmol/l, [3]g/l, [4] M = method of rumen fluid collection, D = diet type.

Table 3 . pH and concentration of metabolites in ruminal fluid aspirated via a cannula, using a syringe (SYR) or a vacuum pump (VAC)[1]

	Diet							
	Concentrate		Grass silage			Significance[4]		
	SYR	VAC	SYR	VAC	sed	M	D	MxD
pH	6.07	6.08	6.55	6.65	0.165	NS	**	NS
Acetic acid[2]	56.7	55.3	40.1	43.3	4.47	NS	**	NS
Propionic acid[2]	29.2	28.2	17.2	18.0	5.81	NS	*	NS
Butyric acid[2]	12.3	12.3	6.7	7.0	1.42	NS	**	NS
Volatile fatty acids[2]	105.0	102.6	66.3	70.9	11.95	NS	**	NS
Acetate : propionate	2.17	2.24	2.79	2.77	0.235	NS	**	NS
Ammonia[3]	89.8	82.3	99.4	107.1	23.6	NS	NS	NS

[1]Mean of samples collected at 5 times for each of 4 animals/diet, [2]mmol/l, [3]g/[4]M = method of rumen fluid collection, D = diet type

NUTRIENT SUPPLY FROM HERBAGE :
In vitro studies

Experiment 7 :

The effect of ensiling on the *in vitro* digestion kinetics of grass cell walls : Unfractionated forage

The water soluble fraction of pre- and post-ensiled forage may differentially influence the rumen environment due to the different concentration and nature of soluble organic acids and proteins in each fraction. *In vitro* techniques allow the digestion of structural carbohydrates to be described when incubated in the presence or absence of the water soluble fraction and thus to determine if ensiling negatively affects the intrinsic rate of structural carbohydrate digestion in the rumen. The objective of this experiment was to determine the effect of ensiling on the digestion of the unfractionated cell wall fraction of perennial ryegrass.

Perennial ryegrass swards were cut on the 5 November and the fresh herbage (G) was precision chopped, pooled and ensiled for 8 weeks, with restrictive (R : 5ml 85% formic acid/kg fresh grass) or extensive (E :15 g sucrose/kg fresh grass)) preservation conditions imposed.

On the day of harvest or silo opening, fresh or ensiled herbages were sampled for chemical analysis. After pooling of herbage or silo contents, a representative sample of the mixed forage was chopped to 1 cm using a paper guillotine. One gram of DM equilvalent was weighed into each fermentation tube and incubated with rumen fluid within 2 h of sampling. Substrates were incubated under nitrogen-excess (N_e) or nitrogen-limited (N_l) conditions. For nitrogen-limited treatments, NH_4HCO_3 was replaced with a molar equivalent of $NaHCO_3$ and casein was omitted. Cultures were sampled in triplicate 11 times over 96 h and residues recovered by vacuum filtration

and washing. Recovered residues were then dried at 40^{O}C for 48 h in an oven with forced air circulation, weighed and ND residue determined.

There was no effect of ensiling on *in vitro* DMD or the digestibile organic matter content of the DM while ensiling decreased the NDF content of the restrictively preserved forage ($p<0.05$) and increased the ADF content of the extensively preserved forage ($p<0.05$, Table 4).

Crude protein and soluble ammonia nitrogen concentrations increased ($p<0.05$) with ensiling with no significant effect on soluble nitrogen . The acid detergent insoluble nitrogen concentration was not affected by preservation. The water soluble carbohydrate (WSC) fraction of grass was reduced by ensiling ($p<0.001$), with the restrictively preserved forage having a greater residual WSC than extensively preserved forage ($p<0.05$). When compared with extensive preservation, the restricted fermentation resulted in lower lactate ($p<0.05$) and VFA concentrations ($p<0.001$). Acetic acid was the predominant VFA formed in both preservation systems, accounting for 98-99% of VFA. The ethanol concentration was not affected by preservation method.
There was no significant interaction between forage type and nitrogen supplementation for any parameter of *in vitro* digestion (Table 5). The rate of NDF digestion was not affected by forage type or nitrogen supplementation. The lag of fermentation was increased by ensiling ($p<0.001$). There was no effect of nitrogen supplementation on the lag of NDF digestion. Ensiling decreased the extent of digestion ($p<0.01$) with the effect most severe for the restrictively preserved forage. Nitrogen supplementation did not affect the extent of digestion.

The apparent extent of digestion (AED) is an estimate of the extent of digestion in the rumen which incorporates the kinetics of digestion measured *in vitro* with an adjustment for the rate of passage from the rumen if the material was actually consumed by a ruminant.

Ensiling ($p<0.001$) and nitrogen supplementation ($p<0.01$) decreased the AED.

It is concluded that ensiling of grass decreased the AED of the cell wall fraction when in the presence of the whole plant which largely reflected an increase in the lag time before digestion commenced.

Experiment 8 :

The effect of ensiling on the *in vitro* digestion kinetics of grass cell walls : Fractionated forage

The objective of this experiment was to determine the effect of ensiling on the apparent digestion of the isolated cell wall fraction of perennial ryegrass.

The fresh and ensiled forage from experiment 7 were dried at 40^{o}C, chopped to 1cm and the aqueous insoluble fraction (F70) prepared as described in Experiment 3. One gram of F70 was weighed into each fermentation tube and incubated with rumen fluid. Substrates were incubated under nitrogen-excess (N_e) and nitrogen-limited (N_l) conditions. Cultures were sampled in triplicate 11 times over 96 h. and residues recovered by vacuum filtration and washing. Recovered residues were dried at 40^{o}C for 48 h and weighed.

There was an interaction ($p<0.05$) between forage type and nitrogen supplementation for the rate of F70 digestion (Table 6). The rate was higher for the restrictively preserved forage when supplemented with nitrogen but lower for the extensively preserved forage ($p<0.05$). There was a significant interaction ($p<0.05$) for the lag of F70 digestion as the lag for grass was higher and the lag for extensively preserved forage was lower when supplemented with nitrogen ($p<0.05$). There was no effect of forage type or nitrogen supplemen-

Table 4. Chemical composition of fresh and ensiled perennial ryegrass

	Component				
	Grass	Restricted	Extensive	Significance	sed
Dry matter (DM) (g/kg)	128.0	134.7	132.7	ns	4.46
Composition of DM (g/kg)					
Crude protein	257.0	267.3	264.7	*	2.06
Neutral detergent fibre	402.0	388.3	398.7	*	3.45
Acid detergent fibre	220.7	228.0	240.3	*	4.27
Acid detergent insoluble N	7.7	9.3	9.0	ns	2.19
Ash	158.7	160.0	165.3	ns	2.96
Water soluble carbohydrate	56.5	33.1	21.3	***	2.53
Total N (TN) (g/kg DM)	41.1	42.8	42.3	*	0.34
Soluble N (g/kg TN)	354.2	483.5	511.2	ns	49.30
NH_3-N (g/kg TN)	1.73	40.3	47.9	***	1.57
Total volatile fatty acids (g/kgDM)	ND	19.9	70.6	***	1.13
Acetate	ND	19.5	69.5	***	1.08
Propionate	ND	UN	1.1		
Butyrate	ND	0.1	0.3	**	0.01
Lactate	3.1	123.2	207.6	***	3.93
Ethanol	ND	33.5	30.4	ns	1.95

[a]ND = not determined, UD = undetectable

Table 5. The effect of forage type and nitrogen supplementation on the *in vitro* digestion of unfractionated cell walls of grass or silage

Forage (F)[a]	Nitrogen (N)[b]	Rate (/h)	Lag (h)	Extent (g/g NDF)	AED (g/g NDF)
Grass	N_e	0.08	5.6	0.93	0.61
	N_l	0.10	7.3	0.91	0.61
Restricted	N_e	0.11	18.7	0.83	0.32
	N_l	0.09	17.7	0.82	0.41
Extensive	N_e	0.05	18.2	0.90	0.39
	N_l	0.07	15.6	0.87	0.44

Main effect	sig	s.e.d	sig	s.e.d.	sig	s.e.d.	sig	s.e.d
F	ns	0.028	***	2.68	**	0.021	***	0.024
N	ns	0.023	ns	2.19	ns	0.017	**	0.024
FxN	ns	0.040	ns	3.79	ns	0.030	ns	0.038

[a]Grass was ensiled under restrictive or extensive ensiling conditions.

[b]N_l refers to the nitrogen-limited treatment where all nitrogen sources in the buffer were omitted, N_e refers to the nitrogen-excess treatment where nitrogen was supplemented.

Table 6. Effect of forage type and nitrogen supplementation on in vitro digestion of isolated cell walls of grass or silage.

Forage (F)[a]	Nitrogen (N)[b]	Rate (/h)	Lag (h)	Extent (g/g NDF)	AED (g/g NDF)
Grass	N_e	0.09	10.8	0.72	0.42
	N	0.09	7.3	0.69	0.45
Restricted	N_e	0.11	8.6	0.77	0.50
	N	0.09	9.1	0.76	0.47
Extensive	N_e	0.07	7.4	0.73	0.50
	N	0.10	12.1	0.68	0.47

Main effect	sig	s.e.d	sig	s.e.d.	sig	s.e.d.	sig	s.e.d
F	ns	0.010	ns	1.17	ns	0.020	***	0.017
N	ns	0.007	ns	0.96	ns	0.017	ns	0.014
FxN	*	0.013	*	1.66	ns	0.028	ns	0.024

[a]Grass was ensiled under restrictive or extensive ensiling conditions.

[b]N_l refers to the nitrogen-limited treatment where all nitrogen sources in the buffer were omitted, N_e refers to the nitrogen-excess treatment where nitrogen was supplemented.

tation on the extent of digestion. Restrictive preservation increased the AED of F70 digestion ($p<0.001$) when compared with grass and extensively preserved forage, and there was no effect of nitrogen supplementation.

It is concluded that ensiling of grass did not negatively affect the digestion of the isolated cell wall fraction.

Experiment 9 :

In vitro ruminal fermentation of the water soluble components of grass or silage

Little information is available on ruminal microbial growth and metabolism on the WSC fraction of grass and how microbial activity is affected by the changes that occur in this fraction during ensiling. The development of equipment to measure cumulative gas production has facilitated measurement of metabolism of soluble substrates by ruminal micro-organisms. The aim of this study was to examine the effect of ensiling *per se* on the anaerobic ruminal fermentation of the soluble component of silage.

Synthetic WSC fractions of fresh (GS) and ensiled forages (SS1) were incubated with clarified rumen fluid (3.0 mg microbial protein (MP)/100ml). A simulated ensiled forage WSC with VFA omitted (SS2) was also used. Gas production (G)), pH, VFA and microbial protein production was monitored over time. For all variables, except for total VFA, there was a significant substrate x time interaction.

The pH remained between pH 6.6-6.9. Grass WSC had a significantly greater and more rapid fermentation, when compared to other substrates after 10h. The VFA concentration at t=0 and t=48 h was 4.3, 60.0, 6.4 and 26.7, 86.5 and 29.2 mmol/l for GS, SS1 and SS2 respectively. The VFA increase over time was expressed as %VFA [VFAt=0 = 100%] to account for initial differences. For % VFA at

48h, GS>SS2>SS1; GS and SS2 were significantly higher than SS1 after 16h, and SS1 did not change over time. Microbial protein concentration increased for GS, SS1 and SS2 (3.0, 3.2, and 2.9, 9.0, 6.8 and 6.3 and 15.0, 8.4 and 7.7 (sed 0.58 and 0.53 for levels of time and substrate) mg/100ml for 0, 8 and 48 h, respectively); SS1 and SS2 did not differ and GS did not increase after 12h. It is concluded that there is a negative impact of ensiling on microbial protein production from the WSC fraction, that high initial VFA concentration inhibited the rate of VFA production but not microbial protein synthesis and that microbial protein production occurs at the expense of VFA production.

Experiment 10 :

The effect of the water soluble fraction pre- and post-ensiling on the *in vitro* digestion of isolated grass cell walls : batch culture study

The results from Experiment 8 and Experiment 9, clarified the consequences of ensiling on ruminal metabolism of the isolated cell wall and water soluble fractions of grass. The aim of this study was to determine the effect of the water-soluble component of perennial ryegrass pre- and post- ensiling on *in vitro* digestion of isolated cell walls.

Fresh grass was precision chopped, pooled and ensiled for 8 weeks in mini-silos, with restrictive (R : 5ml 85% formic acid/kg fresh grass) or extensive (E, 15g sucrose/kg fresh grass) preservation conditions imposed. At harvest or opening of the mini-silos, fresh grass and silages were immediately frozen at - 20^0C for isolation of the water-soluble (W) fraction. While frozen, the herbage was bowl chopped, thawed at 4^0C and the W fraction isolated using a laboratory juice extractor. Structural carbohydrates (F70 fraction) were isolated from each forage as described for Experiment 3. In a series of incu-

bations, the W fraction of grass (W_G), restricted (W_R) or extensively fermented (W_E) forages were added to F70 based on the fresh weight : DM ratio of the original forage. Substrates were incubated under nitrogen-excess (N_e) and nitrogen-limited (N_l) conditions as before. Treatments were sampled in triplicate, 11 times over 96 h and recovered residues were dried at 40^0C for 48 h and weighed.

Ensiling decreased the residual sugar concentration and increased the organic and ammonia nitrogen content of the soluble fraction, with the effects greater for the extensively preserved forage (Table 4).

The rate of digestion of isolated cell walls of grass or R was not affected by any treatment (Table 7a). There was a significant forage by nitrogen interaction ($p<0.05$) for the lag of F70 digestion as the lag of grass was higher and that of R was lower when supplemented with nitrogen ($p<0.05$). Restrictive preservation increased the extent of F70 digestion ($p<0.001$) as did nitrogen ($p<0.05$) and W_G ($p<0.001$) supplementation. There was a lower AED for grass when supplemented with W_R and nitrogen. Otherwise, ensiling increased the AED ($p<0.01$), nitrogen supplementation decreased the AED ($p<0.05$) and supplementation with W_G increased ($p<0.01$) the AED.

There was a significant forage by nitrogen interaction ($p<0.01$) for the rate of digestion of isolated cell walls of grass or E (Table 7b) which reflected a decrease in the rate for E and an increase in the rate for grass due to nitrogen supplementation. There was a lower lag of F70 digestion for E when supplemented with W_G and with nitrogen. This effect was not evident for the F70 of grass. The lag of grass digestion was higher and the lag of E silage was lower when supplemented with nitrogen ($p<0.05$). The extent of F70 digestion was lower when supplemented with W_E compared with W_G. There was a higher AED for the E when supplemented with W_G alone or

Table 7a. The effect of water-soluble fraction (W) and nitrogen supplementation on the *in vitro* digestion of isolated cell walls of grass or restrictively preserved silage

Forage (F)[a]	W[b]	Nitrogen[c]	Rate (/h)	Lag (h)	Extent (g/g F70)	AED (g/g F70)
Grass	W_G	Ne	0.12	10.6	0.65	0.41^{b}
	W_G	N_l	0.10	8.6	0.66	0.43^{ab}
	W_R	Ne	0.11	12.4	0.59	0.35^{c}
	W_R	N_l	0.10	9.2	0.64	0.41^{b}
Restrictive	W_G	Ne	0.09	9.4	0.71	0.44^{ab}
	W_G	N_l	0.11	9.8	0.74	0.47^{a}
	W_R	Ne	0.10	9.3	0.67	0.43^{ab}
	W_R	N_l	0.11	12.0	0.70	0.42^{ab}

Main effect	sig	s.e.d	sig	s.e.d	sig	s.ed	sig.	s.e.d
F	ns	0.009	ns	0.79	***	0.010	**	0.008
W	ns	0.009	ns	0.79	**	0.010	**	0.005
N	ns	0.009	ns	0.79	*	0.010	*	0.007
FxW	ns	0.013	ns	1.11	ns	0.016	ns	0.009
FxN	ns	0.013	ns	1.11	ns	0.016	ns	0.011
WxN	ns	0.013	ns	1.11	ns	0.016	ns	0.009
FxWxN	ns	0.018	ns	1.57	ns	0.021	*	0.014

[a]Grass was ensiled under restrictive ensiling conditions.
[b] W_G refers to the grass WSC fraction and W_R refers to the silage WSC fraction
[c] N_l refers to the nitrogen-limited treatment where all buffer nitrogen sources were omitted, N_e refers to the nitrogen excess treatment where nitrogen was supplemented. Means with similar subscripts are not significantly different ($p<0.05$)

Table 7b. The effect of water-soluble fraction (W) and nitrogen supplementation on the *in vitro* digestion of the isolated cell walls of grass or extensively preserved silage

Forage (F)[a]	W[b]	Nitrogen[c]	Rate (/h)	Lag (h)	Extent (g/g F70)	AED (g/g F70)
Grass	W_G	Ne	0.12	10.6^{b}	0.65	0.41^{bc}
	W_G	N_l	0.10	8.6^{c}	0.66	0.43^{ab}
	W_E	Ne	0.11	11.2	0.63	0.39^{bc}
	W_E	N_l	0.13	9.8	0.61	0.40^{bc}
Extensive	W_G	Ne	0.10	6.2	0.75	0.51^{ab}
	W_G	N_l	0.12	14.8	0.76	0.43^{a}
	W_E	Ne	0.08	13.7	0.67	0.36^{ab}
	W_E	N_l	0.13	14.6	0.64	0.37^{ab}

Main effects	sig	s.e.d	sig	s.e.d	sig	s.ed	sig.	s.e.d
F	ns	0.007	***	0.68	***	0.009	ns	0.007
W	ns	0.007	***	0.68	***	0.009	**	0.009
N	*	0.007	*	0.68	ns	0.009	ns	0.005
FxW	ns	0.010	ns	0.95	**	0.013	*	0.011
FxN	**	0.010	***	0.95	ns	0.013	**	0.00
WxN	ns	0.010	*	0.95	ns	0.013	**	0.010
FxWxN	ns	0.014	***	1.34	ns	0.017	**	0.013

[a] Grass was ensiled under extensive ensiling conditions.

[b] W_G refers to the grass WSC fraction and W_E refers to the silage WSC fraction

[c] N_l refers to the nitrogen-limited treatment where all buffer nitrogen sources were omitted, N_e refers to the nitrogen excess treatment where nitrogen was supplemented. Means with similar subscripts are not significantly different ($p<0.05$)

W_G and nitrogen. The AED of grass and E was lower and higher respectively, when supplemented with nitrogen ($p<0.05$). There was a higher AED for grass when supplemented with W_G rather than W_E and a higher AED when forages were supplemented with W_G and nitrogen rather than W_E and nitrogen.

It is concluded that 1) supplementation with the grass W fraction significantly improved the AED for both ensiled forages when compared with the supplementation of the post-ensiling W fraction 2) these effects were dependent on nitrogen supplementation and were more obvious for the extensively preserved forage.

Experiment 11 :

The effect of the water soluble fraction pre- and post-ensiling on the *in vitro* digestion of isolated grass cell walls : continuous culture study

The objective of this experiment was to assess the importance of the W fraction for grass digestion but to use the RSCC *in vitro* system to alleviate endproduct inhibition. To assess the importance of ensiling on the soluble fraction and subsequent ruminal digestion of NDF, the cell wall fraction was defined as F20 and not the F70 aqueous extract used earlier. To assess the importance of proteolytic alterations during ensiling on subsequent ruminal digestion and MP production, the system was operated under ammonia-excess conditions, with peptide nitrogen supplied only by the experimental treatments.
The cell walls of grass and extensively fermented silage (E)were isolated as described earlier. The simulated W fraction of grass (W_G) contained 1.2g hexose (9.9g fructose, 80.1g glucose and 10g sucrose/100g) and 0.93 casein/10m. The simulated W fraction of the extensively fermented silage had 0.4g hexose, 2.8g lactic acid, 15g ethanol, 0.87g acetic acid, 0.02g propionic acid and 0.5g casein/ 11 ml

Table 8. Operational conditions for the rumen semi-continuous culture and the effect of forage (F[a]) and water soluble fraction (W[b]) on *in vitro* digestibility and microbial protein production.

	Grass		Silage			Significance		
	W_G	W_E	W_G	W_E	s.e.d.	F	W	FxW
Operational conditions								
LDR	5.2	5.4	5.5	5.4	0.26	ns	ns	ns
SDR	1.7	2.3	2.1	2.5	0.03	*	*	ns
Protozoa population (x 10^5)	1.5	1.2	0.9	0.9	2.57	ns	ns	ns
Digestibility (g/kg)								
Dry matter	609	580	569	566	46.8	ns	ns	ns
Neutral detergent fibre	777	759	771	793	51.5	ns	ns	ns
Acid detergent fibre	321	304	277	234	95.3	ns	ns	ns
Crude protein	598	614	561	651	61.6	ns	ns	ns
Estimated rate of digestion	0.018	0.023	0.025	0.022	0.0023	ns	ns	ns
Microbial nitrogen (MN)								
g MN produced/kg DM	8.00	9.70	8.75	8.75	1.04	ns	ns	ns
g MN produced/kg DM digested	16.7	13.2	15.4	15.5	1.77	ns	ns	ns

[a]Perennial ryegrass was ensiled under extensive ensiling conditions. The F20 fraction of each was prepared as described in Experiment 3

[b]Simulated water-soluble carbohydrate composition for Grass(W_G) and silage (W_E) (equivalent to 22.5 g forage DM)

H_2O. The RSCC and its operational conditions were as outlined in Experiment 4, with the following modifications: the buffer solution was supplemented with 0.5 g urea/l buffer and the SDR and LDR were set at 2.5 and 5.0 %/h, respectively. There were two experimental periods of 10 days each. Treatments were randomly assigned to one of four vessels. Two vessels were fed 22.5 g of grass or E silage cell walls every 12 h. For each substrate the two vessels were supplemented with W_G or W_E at every feed on a fresh weight: DM basis.

The LDR did not differ between treatments (Table 8). The biochemical alterations due to ensiling did not influence the cell walll DM, OM, NDF or ADF digestibility *in vitro*.
In this study the supplementation rate was substantially lower than that reported in Experiment 10 as the objective was to replace the nutrient fractions of the W component only. Supplementation therefore did not affect treatment or feed component digestion rates (Table 8). There was no effect of treatment on protozoal numbers or MP production. The MP production was numerically higher when supplemented with W_G, supporting the finding of Experiment 9 that MP production was greater for the grass water-soluble fraction.

It is concluded that (1) ensiling did not affect the DM, NDF, ADF or CP digestibility of the aqueously extracted cell wall fraction of perennial ryegrass (2) supplementation of cell walls isolated before or after ensiling with the soluble fractions pre- and post-ensiling did not influence MP production or forage digestibility.

Experiment 12 :

In vitro investigation of possible negative effects of maturity and ensiling on the kinetics of ruminal digestion of grass cell walls.

Together with ensilage the nutritional potential of herbage can be influenced by maturity, prior to harvesting. The purpose of this experiment was to determine the impact of ensiling on the rumen fermentation characteristics of perennial ryegrass NDF harvested at different stages of growth.

Perennial ryegrass plots were closed on the 17 March 1997 and were cut 7, 10, 12 and 16 weeks thereafter. Grass (G) was ensiled for 8 weeks in mini-silos. Restrictive or extensive ensiling conditions were imposed as before. On the day of harvest or silo opening, fresh forages were chopped to 1 cm and incubated with inoculum, which was previously collected from silage-fed steers, pooled and frozen until required. Incubations were sampled at 0, 1, 3, 8,12, 18, 24, 36, 48, 72 and 96 h (n=3) and the residue remaining at each time point was determined. The extent of fermentation was the average of the 72 and 96 h residues.

All forages were well preserved (mean pH 3.9). The NDF and ADF contents of forages increased with advancing maturity. Ensiling decreased the NDF content of silage *per se* and the ADF content of the extensively preserved silage. Advancing maturity negatively influenced all aspects of ruminal fermentation of NDF. Ensiling significantly increased the NDF lag time (defined as the delay beyond 12 h before active digestion begins), for weeks 12 and 16 and decreased the rate of digestion in the first 24 h of fermentation, for all harvests (Table 9). When compared with the fresh forage, the extent of NDF digestion was decreased in extensively preserved forages for weeks 7 and 12, and increased for week 16. Forages that underwent a restricted fermentation had a lower extent of digestion in week 12

Table 9. Effects of maturity of grass (G), ensiling with sucrose (S) or formic acid (FA) and incubation time on NDF digestion (g/kg) in vitro

Regrowth (week)												
	7			10			12			16		
Time	Forage			Forage			Forage			Forage		
(h)	G	FA	S	G	FA	S	G	FA	S	G	FA	S
0	0	0	0	0	0	0	0	0	0	0	0	0
0												
12	16.4	12.8	10.6	16.4	14.0	10.4	22.5	5.1	3.9	10.2	3.5	2.3
24	50.8	30.9	19.3	32.0	22.1	24.6	31.6	12.1	8.4	18.2	3.5	1.7
48	77.1	67.3	43.3	75.9	69.4	61.7	58.9	55.6	53.1	37.2	18.8	14.7
96	89.0	86.9	71.7	80.1	75.8	75.4	73.2	65.9	58.4	37.7	45.4	48.6

Effect	s.e	sig
Maturity (M)	0.832	***
Ensiling (E)	0.721	***
Time (T)	1.907	***
MxE	1.442	***
TxE	1.907	***
MxT	2.202	***
MxExT	3.815	*

when compared to fresh forage.
Restrictive preservation did not affect the extent of NDF digestion. It is concluded that there is a negative impact of maturity on the pattern of NDF fermentation and that this impact can be decreased by ensiling method.

NUTRIENT SUPPLY FROM HERBAGE :
in vivo studies

Experiment 13.

Influence of supplement carbohydrate degradability on growth of silage-fed beef cattle and on ruminal metabolism in lambs

To clarify the impact of the rate of ruminal digestion of supplementary energy on growth of beef cattle, 56 Friesian steers (bodyweight = 338 kg) were penned in groups of 7, offered grass silage *ad libitum* and 3.5 kg/head daily of one of four isoenergetic and isonitrogenous concentrates formulated with varying combinations of carbohydrates with fast (sugar), medium (starch) and slow (digestible fibre) degradability. The major ingredients were: CP0 (0 g citrus pulp (CP) and 920 g barley (B)/kg), CP26 (265 g CP and 616 g B/kg), CP56 (556 g CP and 282 g B/kg) and CP80 (803 g CP and 0 B/kg). The starch and sugar concentrations (g/kg) of CP0, CP26, CP56 and CP80 were 458 and 28, 291 and 87, 171 and 139 and 66 and 183, respectively. When wincubated with ruminal fluid *in vitro*, the rate of fermentation increased ($P<0.05$) with level of CP inclusion in the concentrate (e.g. 34, 49, 54 and 56 (s.e.d. 0.75) ml gas was produced after 2.75 h, and 135, 159, 163 and 172 (s.e.d. 1.80) ml gas was after 6h incubation of CP0, CP26, CP56 and CP80, respectively). Steers were weighed on consecutive days at the beginning and end of the study which lasted approximately 16 weeks. Bodyweight gain was unaffected by levels of citrus pulp inclusion (106, 105, 94 and 102 kg (s.e.d. 8.0) for CP0, CP 26, CP 56 and CP 80 respectively). To allow adjustment for possible con-

founding effects of differences in the weight of gut contents, the steers were also weighed at the beginning and end of the study after feed had been withdrawn for 48 hours. Mean fasted bodyweight gain was 112, 109, 98 and 104 kg (s.e.d. 7.5) for CP0, CP26, CP56 andCP 80 g/kg citrus pulp, respectively.

Thirty wether lambs were offered a ryegrass *(Lolium)* silage (S1: DM 181 g/kg, pH 4.0, crude protein 171 g/kg DM and DM digestibility 775 g/kg) alone or with each of the concentrates (390 g/kg DM) at 18 g DM/kg bodyweight. After 14 days adaptation, urine was collected for 7 days. Sheep were then offered a second ryegrass silage (S2; DM 204 g/kg, pH 4.1 crude protein 163 g/kg and DM digestibility 701 g/kg) alone or with each of the concentrates and urine was again collected. Animal data were analysed according to a split-plot design with ration type in the main plot and silage type in the sub-plot and are summarised in Table 10.

It is concluded that silage type had a greater effect than the rate of concentrate fermentation on ruminal microbial protein synthesis.

Experiment 14 :

Nitrogen balance and ruminal microbial protein supply in lambs offered grass or grass/clover diets

The objectives of this study were to measure ruminal microbial protein synthesis and nitrogen balance in young ram lambs, chosen as a model for ruminants with a high protein requirement (n = 10/treatment), offered grass (*Lolium perenne,* cultivar Majella) or a grass/clover (*Trifolium repens,* cultivar Susi) mixture harvested in June, August and October. Separate groups of animals were used in each phase of the study which consisted of 14 days adaptation followed by a 10 day measurement period. Feeding level was fixed at 18g dry matter (DM)kg bodyweight. Grass and grass/clover swards received

Table 10. Purine derivative excretion and estimated microbial nitrogen supply in sheep

Silage (S)	Silage 1					Silage 2						Significance		
Conc (C)	None	CP0	CP26	CP56	CP80	None	CP0	CP26	CP56	CP80	sed	C	S	CxS
Bodyweight (kg)	37.7	39.3	37.8	38.8	38.8	38.5	43.3	41.5	44.4	41.5	1.39	**	NS	NS
Purine derivative excretion (mmol/d)														
Allantoin	10.6	12.6	14.9	11.6	13.7	12.6	10.7	9.9	10.6	11.7	1.79	NS	*	0.07
Uric acid	0.9	0.6	0.5	0.8	0.6	0.6	0.3	0.3	0.4	0.3	0.11	*	**	NS
Xanthine + hypoxanthine	3.5	2.7	2.4	2.6	2.5	2.7	1.5	1.3	1.4	1.3	0.25	*	***	NS
Total	15.0	15.9	17.9	15.0	16.8	15.8	12.4	11.4	12.4	13.4	1.75	NS	***	0.06
Microbial nitrogen (g/day)	13.0	13.8	15.5	13.0	14.6	13.7	10.8	9.9	10.8	11.6	1.51	NS	**	0.06

50 and 0 kg N/ha, respectively, prior to the 6 week regrowth interval for each harvest. Grass represented 963, 993 and 993 g/kg herbage DM in the grass sward in measurement phases 1, 2 and 3, respectively. Corresponding values for clover in the grass/clover sward were 70, 261 and 369 g/kg herbage DM. The average *in vivo* DM digestibility of the grass was 765, 758 and 772 g/kg in measurement phases 1, 2 and 3, respectively. The corresponding values for the grass/clover sward were 795, 768 and 797 g/kg. Nitrogen balance data are summarised in Table 11. Allantoin excretion and estimated microbial nitrogen flow to the small intestine data are summarised in Table 12.

It is concluded that harvesting time had a bigger impact on nutrient supply from herbage than sward type and that increasing clover content in the herbage decreased the biological value (g retained/kg absorbed) of dietary protein. This probably reflects changes in the fraction of dietary protein undergraded in the rumen.

RATION FORMULATION ON COMMERCIAL FARMS

Experiment 15 :

Feeding strategies and systems used by efficient winter finishers to maximise animal performance and minimise feeding costs

The objectives of this study were to monitor growth of cattle on selected, efficient, winter finishing units and to relate this to the type, quantity and quality of feeds consumed by the animals. The specialised winter finishing units were chosen based on the following criteria: (1) engaged in this system of beef production for 5 years or more, (2) finish 90 cattle or more, and (3) this is the predominant

Table 11. Nitrogen balance in lambs offered grass or grass/clover harvested at three times during the growing season

Harvest time (H)	June		August		October			Significance		
Sward (S)	Grass	Grass/ clover	Grass	Grass/ clover	Grass	Grass/ clover	sed	S	H	SxH
Bodyweight (kg)	3.76	37.8	35.4	36.0	28.9	28.5	1.67	NS	***	NS
Nitrogen balance (g/day)										
Intake	47.4	16.6	19.4	18.2	13.5	16.2	0.92	NS	***	**
Loss										
Faeces	4.0	4.0	3.9	4.4	3.0	3.0	0.30	NS	***	NS
Urine	8.6	6.7	8.7	8.9	6.6	904	0.53	NS	*	***
Total	12.6	10.8	12.6	13.3	9.6	12.4	0.76	NS	**	***
Retention (g/day)	4.8	5.8	6.8	4.9	3.9	3.8	0.41	NS	***	***
(g/kg $W^{0.75}$)	74	88	100	73	49	48	7.6	NS	***	***
(g/kg intake)	277	350	350	270	288	238	19.8	0.1	***	***
(g/kg absorbed)	359	463	438	356	369	290	23.0	NS	***	***

Table 12. Allantoin excretion and estimated microbial protein supply in lambs offered grass or grass/clover harvested at three times during the growing season

Harvest time (H)	June		August		October			Significance		
Sward (S)	Grass	Grass/ clover	Grass	Grass/ clover	Grass	Grass/ clover	sed	S	H	SxH
Allantoin (mg/day)	918	911	964	966	798	804	62.5	NS	**	NS
Small intestinal flow										
Purines (μmol/kg $W^{0.75}$)	520	514	564	559	551	555	24.8	NS	*	NS
Microbial-N (g/day)	7.30	6.88	7.06	7.03	5.72	5.92	0.424	NS	***	NS

Table 13. Ration ingredient composition (g/kg dry matter) for winter finishing cattle

	Farm Code									
	A	B	C	D	E	F	G	H	I	J
Grass silage	490	299	202	393	412	209	298	519	383	420
Fodder beet	396	---	396	236	255	---	398	56	319	436
Sugar Beet	---	---	---	---	---	332	---	---	---	---
Rapeseed meal	57	---	---	---	---	---	---	---	63	---
Soyabean meal	57	17	128	---	48	---	---	---	63	---
Soda wheat	---	146	---	---	---	---	---	---	172	---
Molasses	---	43	77	---	38	44	---	---	---	---
Potatoes	---	135	---	---	---	---	---	---	---	---
Barley	---	96	---	112	88	146	152	172	---	---
Maize meal	---	87	---	---	---	---	---	---	---	---
Straw	---	9	33	24	10	---	41	---	---	20
Hay	---	---	---	---	---	---	---	7	---	---
Brewers grains	---	168	---	---	---	---	---	---	---	---
Wheat	---	---	156	---	88	---	---	---	---	---
Urea	---	---	8	---	---	---	---	---	---	---
Citrus pulp	---	---	---	116	---	151	---	---	---	---
Cotton seed	---	---	---	119	---	118	---	---	---	---
Sugar beet pulp	---	---	---	---	61	---	---	---	---	---
Protein mix	---	---	---	---	---	---	112	---	---	---
Sugar pressed pulp	---	---	---	---	---	---	---	126	---	---
Distillers grains	---	---	---	---	---	---	---	120	---	-

Table 14. Growth performance of winter-finishing cattle

Item	Minium	Maximum	Mean	Co-efficient of vari. (%)
Initial liveweight (kg)	575	772	631	12.3
Daily gain (kg)	0.73	1.06	0.90	12.0
Carcass weight (kg)[1]	348	489	419	13.2
Kill-out (%)[1]	55.0	57.0	56.3	1.8
Dry matter (DM) intake				
(kg/day)	8.8	14.5	11.0	14.0
(g/kg liveweight)	14.8	19.2	16.7	9.0
Feed conversion efficiency (kg DM/kg gain)	9.5	15.1	12.6	15.4
Ration cost (p/kg DM)	8.8	11.7	9.9	8.8
Cost of gain (£/kg)	0.86	1.71	1.23	21.7

[1]Based on 6 farms

livestock enterprise. On each farm, a minimum of 50 cattle were identified, tagged where necessary and background information, i.e. age, breed, source, etc., collected. After animals had been housed and adapted to their winter ration, they were weighed on consecutive days and again as close as was practicable to slaughter. On two occasions, feed consumption was monitored during a 7-day period on each farm. All selected farms used a feeder-wagon which was "validated" prior to measurement of feed consumption.

The variation in ration composition on the 10 farms is summarised in Table 13 and animal performance in Table 14.

In summary, diverse strategies were used on the 10 farms, i.e. purchase weight of the cattle, finishing period, ration composition, housing, etc. Considerable variation in feed intake and animal growth was observed between farms. Average animal performance on individual farms was not better than would be typically recorded in a research environment. There is scope on many of the farms to improve technical performance and to decrease the costs of production.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the considerable contribution of many colleagues to this project, in particular the technical, farm and clerical staff at Grange Research Centre. Aiveen Marron and Vincent McHugh, together with the staff of Grange Laboratories and other technical staff at Grange provided a vital, skilled contribution. Similarly, Grange farm and service staff willingly undertook considerable work in the animal-related research in this project. Mary Smith and Ann Gilsenan always provided clerical support to a very high standard.

Many parts of the work in this report involved fruitful collaboration with other Teagasc research and advisory colleagues, as well as with colleagues from University College Dublin (Dr. F. O'Mara, Dept. Animal Science and Production; Dr. E. Doyle, Dept. Industrial Microbiology) and Dublin City University (Dr. M. O'Connell, Biological Sciences).

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