End of project report

Quantification of nutrient supply in forage-based diets for beef cattle

Grange Beef Research Centre

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Quantification of nutrient supply in forage-based diets for beef cattle

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Introduction

Cattle rearing systems in Ireland are predominantly grass-based as 80% of agricultural land is dedicated to grassland (silage, hay and pasture) (CSO, 2007). Feed costs represent the largest single variable cost in beef production in Ireland.

Grazed grass is generally the cheapest source of food available for beef (and milk) production provided that the environment and management permit high yields of high quality herbage to be utilised (McGee, 2000). Environmental legislation and the rules of environmental schemes such as the European Union (EU) Rural Environmental Protection Scheme are progressively restricting the application of fertilizer Nitrogen (N), and many grazing systems are becoming more extensive.

Over 80% of all farms in Ireland make grass silage (O'Kiely et al., 1998) and it accounts for 87% of total grass conserved (Mayne and O'Kiely, 2005). The deficiencies in nutrient supply to beef cattle from grass silage are usually overcome by supplementing with concentrates (McGee, 2005), which are primarily cereal-based (Drennan et al., 2006). However, diverse types of concentrates containing a variety of feed ingredients, particularly non-cereal by-products are available and frequently fed as supplements to grass silage or as high-concentrate diets. The relatively small amount of information available on feeding these contrasting concentrates to beef cattle is inconsistent. Moreover, there has been an increased use of other ensiled forages such as maize and whole-crop cereals. These forages have high intake potential and can reduce the concentrate feeding level, while maintaining or increasing performance of beef cattle (Keady, 2005).

With increasing costs of beef production and increasing constraints of environmental regulations, efficient utilisation of consumed nutrients by cattle is imperative in providing sustainable production and income to farmers.

Feed evaluation systems are used to match the dietary nutrient supply with animal requirements for a specific level of production (Dijkstra et al., 2007). These systems are important in order to optimise the efficiency of feed utilisation, to improve animal performance and to reduce nutrient losses to the environment (Dijkstra et al., 2007). Although the reticulo-rumen is central to the profile of nutrients available for absorption, yet quantitative knowledge of the rates of passage and the digestion of nutrients in the rumen is limited compared with that on degradation rates (Dijkstra et al., 2007). There is a lack of information that adequately characterises the supply of nutrients from forages and feedstuffs specific to Ireland, especially for fresh grass-based diets of which, there are very few studies reported in the literature. This shortcoming impedes our ability to capitalise on the merits of evolving feeding systems.

This project aimed to:

1. Increase the knowledge and advance the understanding on rumen digestion and nutrient flow from the rumen of the main forages / forage-based diets offered to beef cattle in Ireland.

2. Evaluate strategies for optimal utilization of nutrients consumed by cattle.

Abbreviations

- ADFom, acid detergent fibre expressed exclusive of residual ash;
- aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash;
- AIA, acid insoluble ash;
- BW, bodyweight;
- CP, crude protein;
- D, potentially degradable fraction;
- DM, dry matter;
- DMI, dry matter intake;
- DMD, dry matter digestibility;
- DNDFom, digestible NDF expressed exclusive of residual ash;
- DOMD, digestible organic matter in the dry matter;
- ED, effective degradability;
- INDFom, indigestible NDF expressed exclusive of residual ash;
- $k_{d,}$ fractional rate of digestion in the rumen;
- k_i, fractional rate of intake;
- $k_{p,}$ fractional rate of passage from the rumen;
- MN, microbial N;
- NDFom, neutral detergent fibre expressed exclusive of residual ash;
- N, nitrogen;
- NAN, non-ammonia nitrogen;
- NANMN, non-ammonia non-microbial nitrogen;
- $NH_{3,}$ ammonia;
- OM, organic matter;
- OMAD, OM apparently digested in the rumen;
- OMTD, OM truly digested in the rumen;
- PDI, protein truly digestible in the small intestine;
- PDIE, true protein absorbable in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen;
- PDIN, true protein absorbable in the small intestine when degradable N is limiting microbial protein synthesis in the rumen;
- RI, regrowth interval;
- SAS, Statistical Analysis Systems;
- U, undegradable fraction;
- VFA, volatile fatty acids;
- W, instantly degradable fraction; WSC, water soluble carbohydrate;

EXPERIMENT 1: Rumen fermentation, microbial protein synthesis, and nutrient flow to the omasum in cattle offered corn silage, grass silage, or whole-crop wheat

1. Introduction

Beef cattle rarely consume sufficient grass silage (**GS**) to achieve their production potential, and as a result energy-rich concentrates are routinely supplemented in practice (McGee, 2005). Alternative ensiled forages are limited in many parts of Northern Europe, but recent advances in plant breeding, agronomic practices, and forage conservation technologies have led to an increase in the use of maize and whole-crop silages for feeding beef cattle.

Previous studies have generally reported that feeding corn silage (**CS**) either ad libitum or as a mixture in grass silage-based diets increased DMI and performance of beef cattle (O'Kiely and Moloney, 1995, 2000; Keady and Kilpatrick, 2004) and early lactation dairy cows (Burke et al., 2007). In contrast, several studies have shown an improved DMI without an increase in performance when feeding whole-crop wheat (**WCW**) silages preserved by fermentation or urea-treatment to beef cattle (O'Kiely and Moloney, 1995, 2000; Keady et al., 2007) and early lactation dairy cows (Burke et al., 2007). Furthermore, other studies have found no difference in the performance of beef cattle offered fermented or urea-treated WCW silages, despite a higher DMI of the latter (O'Kiely and Moloney, 1999; Walsh et al., 2005). Whole-crop silage and CS are a less homogenous feed than GS because proportions of grain to straw can vary substantially.

To better explain the responses of beef cattle offered corn and WCW silages, a more comprehensive understanding of the effects of these forages on ruminal metabolism and digestion is required, but currently only limited data are available. The objectives of this experiment were to determine the effect of forage source; GS, CS, fermented WCW (**FWCW**), or urea-treated processed WCW (**UPWCW**), on forage intake, ruminal fermentation, microbial protein synthesis, some plasma metabolites, nutrient flows to the omasum, and ruminal and total tract digestibility in cattle.

2. Materials and Methods

Silage Preparation

The unwilted GS was harvested from a primary growth sward, which consisted mainly of perennial ryegrass (*Lolium perenne* L., cv. Millenium) on 6 June. It was mown with a rotary mower (Pottinger conditioner mower, Pottinger, Grieskirchen, Austria) and harvested at 15.1% DM with a precision-chop harvester (Pottinger Mex VI, Pottinger, Grieskirchen, Austria). A formic acid-based additive (Add-SafeR Interchem Ltd., Cherry Orchard Ind. Est., Dublin 10, Ireland) was applied at a rate of 2.5 L/t of grass. A commercial crop of winter wheat (*Triticum aestivum* L., cv. Soissons) was harvested on 7 August at 41.0% DM and ensiled without additive to provide FWCW. The remainder of the crop was harvested on 3 September at 66.6% DM and a urea + urease additive ('Home 'n' Dry', Volac, Royston, UK) was mechanically applied evenly throughout the forage at a rate of 48.5 kg/t of forage DM. The FWCW and UPWCW were harvested to the same stubble height. The corn (*Zea Mais*. L., cv, Benicia) was grown under plastic mulch, harvested on 6 October at 30.4% DM, and ensiled without additive. A precision chopper (Class Jaguar 860 series, Claas, Bury St Edmonds, UK) was used to collect the CS and WCW, with only the grain in the CS and UPWCW mechanically broken up during collection. All silages were stored in bunker silos (18 × 4.6 × 2.4 m) and compacted (JCB 412S, Rocester Staffordshire, England; 7,800 kg) to ensure expulsion of air before sealing with 2 layers of black polythene sheeting and weighted with tires.

Experimental Design and Animal Management

Four Holstein-Friesian steers fitted with rumen cannulas and with mean BW 509 (SD 6.3) kg were used in the experiment. The 4 steers were offered the 4 forages (GS, CS, FWCW, or UPWCW) in a Latin Square design of 4 periods, each of 21 d, with 13 d for dietary adaptation followed by 8 d for sampling. The 4 experimental forages were supplemented with 3.0 kg of concentrate daily. The concentrate consisted of rolled barley (650 g/kg), soybean meal (280 g/kg), molasses (50 g/kg), and minerals plus vitamins (20 g/kg). The concentrate was formulated to supply a minimum of 110 g CP/kg of diet DM when fed with the CS. The forages were offered for ad libitum intake during the days used for adaptation, and thereafter at 0.90 of average forage DMI, calculated from the last 3 d of adaptation period for each individual animal. During the whole experiment the forages were fed in 2 daily meals at 0830 and 2030. The 3.0 kg of concentrate was also offered twice daily at 0830 and 2030 in 2 equal meals until d 13 and then at 0.90 of their allocation until the end of each period. Animals were housed in individual stalls and had access to water at all times. All procedures in the study were conducted under experimental licence from the Irish Department of Health and Children in accordance with the

Cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulation, 1994.

Measurements

Forages and concentrates offered were sampled daily during the collection period. Forage refusals were weighed and sampled daily during d 11 to 21. All samples were stored at -20°C. Forage samples were pooled for 2-d intervals from d 11. The concentrate samples from each of the 4 periods were pooled for d 11 to 13 and 14 to 21 giving 2 concentrate samples per period. Rumen fluid samples of approximately 200 mL were collected through the rumen cannulae according to Moloney and Flynn (1992) at 0830, 1030, 1230, 1430, 1630 and 1830 on d 14 of the 4 periods. Rumen fluid pH was measured immediately after collection using an Orion digital pH meter (Orion SA 720; Thermo Fisher Scientific, Waltham, MA, USA). A 20-mL sub-sample was preserved with 0.5 mL of 9 *M* sulphuric acid and stored at –20°C for subsequent analysis of ammonia, VFA, and lactic acid.

The markers used to estimate digesta flow from the rumen were indigestible NDF (**INDF**) for the large particle phase (Huhtanen et al., 1994), Yb-acetate for the small particle phase (Siddons et al., 1985b), and Co-EDTA for the fluid phase (Uden et al., 1980). Following a priming dose of 2.5 g of Yb-acetate and 8.0 g Co-EDTA dissolved in 1 L of distilled water, constant rates of 2.5 g of Yb and 8.0 g of Co dissolved in 4 L of distilled water were infused into the rumen using a multi-channel peristaltic pump (Watson Marlow, 502 S, Falmouth, UK) from d 14 to 20. The volumes of liquid infused daily (~ 4 L) were recorded. To determine digesta flow, 400 mL spot samples were collected from the omasal canal twice daily using an alternating pressure - vacuum system as described by Huhtanen et al. (1997) and modified by Ahvenjärvi et al. (2001). Two samples per day were collected at 6 h intervals for d 17 to 20. Sampling started at 0830 on d 17 and advanced 90 min each day such that samples represented each 90 min between the morning and evening feed. Omasal digesta samples were kept on ice and then stored at - 20°C. At the end of each period, these omasal digesta samples were thawed at room temperature and then pooled on an equal volume basis over the sampling times. Digesta was separated into 3 phases according to Ahvenjärvi et al. (2000) whereby the digesta was first squeezed through 2 layers of cheesecloth. Digesta retained on the cheesecloth was termed the large particle phase. The remaining filtrate was centrifuged at 10,000 x *g* for 15 min, the supernatant was aspirated and termed the liquid phase, whereas the residual particles were termed the small particle phase. Large and small particle and liquid phase samples were frozen, freeze dried, and ground through a 1-mm screen prior to chemical analysis of Co, Yb, INDF, ash, N, NDF, ADF, and starch. The true digesta flows into the omasum were calculated according to the triple marker method (France and Siddons, 1986). Reticulorumen samples of 500 mL were obtained manually once daily according to the method of Ahvenjärvi et al. (2002) at 1730, 1430, 0830 and 1130 on d 17, 18, 19, and 20, respectively for determination of microbial protein synthesis. The microbial pellet was prepared according to the method of Ahvenjärvi et al. (2000). Microbial nitrogen (**N**) flow was determined using purine bases as a microbial marker. Fecal grab samples of approximately 500 g were collected parallel with the omasal digesta sampling from every animal in each of the 4 periods (Ahvenjärvi et al., 2001). These samples were stored at -20°C. At the end of the experiment these samples were thawed and pooled on an equal weight basis, per animal, per period. One sub-sample of composited feces per animal was dried at 98°C for 48 h to determine DM, whereas another sub-sample was dried at 60°C for 48 h and ground through a 1-mm screen for the analysis of Yb, Co, INDF, ash, N, NDF, AIA, and starch.

On d 21, 2 blood samples were obtained by jugular venipuncture from each animal immediately before the morning feeding and 3 and 6 h after the morning feeding. Blood was collected into a 10 ml and 4 ml evacuated tube containing lithium heparin and sodium fluoride-EDTA K3, respectively, as anticoagulants. Blood samples were then centrifuged (2,500 × *g*, 20 min, 4°C) and the plasma was stored at -20°C prior to analysis of urea, glucose, and beta hydroxybutyrate.

Chemical analysis

The DM content of forages and concentrates was determined after drying at 85°C for 24 h and 16 h, respectively, in an oven with forced air circulation. Forage DM content was corrected for volatile losses as described by Porter and Murray (2001). Composited forage and concentrate samples were oven dried at 40°C for 48 h and then ground through a 1-mm screen (Willey mill; Arthur H. Thomas, Philadelphia, PA) for analysis of IVDMD (Tilley and Terry, 1963), for NDF using amylase and sequentially for ADF (Van Soest et al., 1991), ash content by combustion at 550°C for 5 h, total N using a combustion assay (Leco FP-428; Leco Instruments, Inc., St Joseph, MI), water soluble carbohydrates (WSC) (anthrone method; Thomas, 1977), starch (McCleary et al., 1997), and AIA (Van Keulen and Young, 1977). Rumen fluid and silage extracts were analysed for ammonia (measured using the Olympus AU400 and the Thermo Electron Infinity Ammonia Liquid Stable Reagent kinetic method) and lactic acid (using the Olympus AU400 and the L-Lactic Acid UV-method test kit; Boehringer

Mannheim / R-Biopharm catalogue number 10139084035) with the enzyme D-Lactate Dehydrogenase (Boehringer Mannheim / R-Biopharm catalogue number 10106941001). The concentration of VFA (acetic, propionic, isobutyric, n-butyric, isovaleric and n-valeric) were measured in the rumen and omasal fluid and in the silage extracts using an automated gas chromatograph (Shimadzu Gas Chromatography GC-8A; Shimadzu Corporation, Kyoto 604-8511, Japan; Brotz and Schaefer, 1987).

Omasal digesta and feces were analysed for Yb and Co according to Hart and Polan (1984). The INDF concentration of forages, small and large omasal particle phases, and feces were determined according to Ahvenjärvi et al. (2000) whereby between 2 to 4 g samples were incubated, in duplicate, in the rumen of 2 cattle for 12 d in 6-µm polyester bags (Sefar Inc., Ruschlikon, Switzerland). Following rumen incubation, bags were rinsed in cold water using a household washing machine for 25 min and boiled in NDF solution for 1 h, rinsed, and dried to a constant weight at 60^{o}C. The remaining residue was ignited at 600^{0}C for 18 h to determine the OM content of indigestible residue. Purine bases in the microbial pellet were determined according to the method of Zinn and Owens (1986) and modifications of Makkar and Becker (1999).

Plasma urea, beta-hydroxybutyrate and glucose concentrations were determined on an automated analyzer (Olympus AU 400, Japan) using the reagents supplied by Olympus.

Statistical Analyses

Data were analyzed using the mixed procedures of SAS (SAS, 2003) for a 4 × 4 Latin square design according to the following statistical model:

$Y_{ijk} = \mu + A_i + P_j + D_k + e_{ijk}$,

where μ is the overall mean, A_i is a random effect of animal, and P_j and D_k are the fixed effects of period and diet, respectively. All reported values are least square means, which were separated using the PDIFF option in SAS.

Rumen fermentation parameters and plasma metabolites were analyzed as repeated measures with the model:

$Y_{ijk} = \mu + A_i + P_j + D_k + T_l + D_k \times T_l + e_{ijkl}$

where μ, A_i, P_j, D_k are as previously described; T_l is the sampling time; and $D_k \times T_l$ is the interaction between diet and time.

3. Results

Feed Composition

The mean chemical composition of the forages and concentrate used in this study are shown in Table 1.1. The GS was characterised by having a relatively low DM, moderate IVDMD, high NDF, and low CP. Compared to GS the alternative forages underwent a more restricted fermentation as evidenced by the lower concentrations of fermentation acids and ethanol. The evidence is that little fermentation occurred with the UPWCW. Of the alternative forages, CS had the numerically highest IVDMD and the lowest DM, CP, and fiber concentrations, whereas UPWCW had the highest DM, CP, and fiber concentrations and the lowest IVDMD. Starch concentrations in the alternative forages were highest in CS and lowest in FWCW.

Feed Intake

Forage, concentrate and total daily DMI are presented in Table 1.2. Forage and total DMI were lower ($P < 0.05$) for GS than the other diets, which did not differ from one another (P greater or equal 0.12).

Rumen Fermentation

Mean rumen fluid pH ($P = 0.07$) and D ($P = 0.15$) and L-lactate ($P = 0.11$) and valeric acid ($P = 0.05$) concentrations did not differ among forages (Table 1.3). Rumen concentration of ammonia N was highest for UPWCW and lowest for CS ($P < 0.001$). Total VFA concentrations were higher ($P < 0.05$) for CS than GS and UPWCW, with FWCW being intermediate. Molar proportion of butyric acid was highest for CS and lowest for GS ($P < 0.001$). Molar proportion of acetic acid was lower ($P < 0.01$) for CS than the other forages, which did not differ (P greater or equal 0.34). Molar proportion of propionate was higher ($P < 0.05$) for GS and CS than UPWCW, with FWCW being intermediate. Acetate to propionate ratio was higher ($P < 0.05$) for UPWCW than the other forages, which did not differ.

OM, NDF, and Starch Omasal Flow and Digestibility

Organic matter intake ($P < 0.01$) and omasal OM flow ($P < 0.05$) were lower and OM apparent ruminal digestibility was higher ($P < 0.05$) for GS than the other forages, which did not differ (Table 1.4). Apparent total tract OM digestibility was higher ($P < 0.05$) for GS and CS than FWCW, with UPWCW being intermediate. Intake of NDF did not differ ($P = 0.78$) between the forages, but omasal NDF flow was higher for FWCW ($P < 0.01$) and UPWCW ($P < 0.05$) than GS, with CS being intermediate. Apparent ruminal digestibility of NDF and the amount of NDF digested in the rumen (kg/d) was higher ($P < 0.01$) for GS compared to the other diets, which did not differ. Total tract NDF digestibility was higher ($P < 0.05$) for GS than CS and UPWCW which in turn were higher than FWCW. Ruminal NDF digestibility, as a proportion of total tract digestibility, was lower ($P < 0.05$) for UPWCW than GS, CS, and FWCW, which did not differ (P greater or equal 0.06)

Starch intake was higher ($P < 0.001$) for CS, FWCW and UPWCW than GS. Apparent ruminal starch digestibility ($P = 0.88$) and ruminal starch digestibility, as a proportion of total tract digestibility ($P = 0.86$), did not differ between the diets. The amount of starch digested in the rumen and total tract digestibility of starch were higher ($P < 0.05$) for CS than FWCW with UPWCW intermediate and GS lowest ($P < 0.01$).

Nitrogen Metabolism

The N intake, omasal flows of N components, and rumen and total tract digestibility of N for the diets are presented in Table 1.5. Nitrogen intake was higher for UPWCW than FWCW, which in turn was higher than GS, with CS being intermediate ($P < 0.001$). Omasal N and non-ammonia nitrogen flows were higher ($P < 0.01$) for CS, FWCW, and UPWCW than GS. When N flow was expressed as a proportion of N intake, CS was higher than GS ($P < 0.01$) and UPWCW ($P < 0.05$), and FWCW was higher ($P < 0.05$) than GS. Microbial N flow was lower for GS compared to CS ($P < 0.05$) and FWCW ($P < 0.01$), and was also lower ($P < 0.05$) with UPWCW compared to FWCW. Flow of non-ammonia non-microbial N was higher ($P < 0.05$) for UPWCW than FWCW and GS, with CS being intermediate. Efficiency of microbial N synthesis in the rumen was higher for FWCW compared to CS ($P < 0.05$) and GS ($P < 0.01$), with UPWCW being intermediate. Apparent ruminal N digestibility was higher for GS than CS ($P < 0.01$) and FWCW ($P < 0.05$), and was also higher for UPWCW compared to CS ($P < 0.05$). Apparent total tract digestibility tended to be higher ($P < 0.1$) for GS relative to the other forages.

Blood Metabolites

Blood composition data are presented in Table 1.6 as mean values of the 3 samples taken throughout the day. Animals fed CS had the highest ($P < 0.001$) concentrations of plasma beta-hydroxybutyrate, whereas concentrations were lowest ($P < 0.001$) for GS with FWCW and UPWCW intermediate and not differing from each other ($P = 0.24$). Plasma urea concentrations were highest for UPWCW and lowest for CS ($P < 0.001$) with GS and FWCW intermediate and not differing from each other ($P = 0.52$).

4. Conclusions

Feeding CS, FWCW, and UPWCW to beef cattle increased DM and N intake and N flow entering the omasal canal and decreased OM and NDF ruminal digestibility when compared to GS. Intake was similar but total tract digestibility of OM, NDF, and starch was higher for CS than FWCW. Microbial N flow was higher for CS and FWCW compared to GS but only FWCW increased the efficiency of microbial N synthesis. The UPWCW had a higher total tract digestibility of NDF and lower flow of microbial N to the omasal canal than FWCW. Feeding alternative forages to GS can significantly increase feed DMI and alter rumen fermentation and site of nutrient digestion when offered to cattle supplemented with 3 kg concentrate daily.

Table 1.1 Chemical composition (g/kg DM unless otherwise stated) of the 4 forages and concentrates fed to steers (± SD)[1]

Item	GS	CS	FWCW	UPWCW	Concentrate
DM, g/kg	191 ± 10.6	318 ± 9.3	412 ± 13.3	725 ± 16.6	834 ± 2.3
IVDMD	712 ± 22.8	754 ± 15.6	692 ± 11.9	699 ± 31.9	889 ± 6.9
IVDOMD	633 ± 22.9	723 ± 16.1	655 ± 13.2	672 ± 32.2	839 ± 7.1
Ash	95 ± 8.1	37 ± 2.6	53 ± 4.5	48 ± 7.5	50 ± 3.5
CP	112 ± 5.0	84 ± 5.6	112 ± 5.4	159 ± 11.5	204 ± 11.8
NDF	620 ± 11.2	393 ± 30.0	428 ± 23.4	437 ± 46.2	111 ± 26.1
Indigestible NDF	100 ± 39.4	84 ± 13.8	135 ± 11.3	119 ± 22.5	38 ± 12.6
ADF	385 ± 10.1	226 ± 15.9	248 ± 21.9	268 ± 34.7	63 ± 8.4
Starch	ND[2]	307 ± 19.4	246 ± 21.8	276 ± 36.1	373 ± 18.1
pH	4.20 ± 0.23	4.00 ± 0.23	4.14 ± 0.23	7.25 ± 0.47	ND
Lactic acid	91 ± 19.4	65 ± 12.6	27 ± 5.0	3 ± 1.7	ND
Acetic acid	31.8 ± 5.4	14.6 ± 2.66	12.8 ± 3.90	8.3 ± 2.7	ND
Propionic acid	0.5 ± 1.4	0.5 ± 0.42	0.6 ± 0.36	0.3 ± 0.07	ND
Butyric acid	2.0 ± 1.2	0.5 ± 0.41	1.0 ± 1.02	0.5 ± 0.30	ND
Ethanol	17.9 ± 8.8	5.9 ± 2.25	2.8 ± 0.88	0.4 ± 0.13	ND
NH_3, g/kg of total N	134 ± 13.9	73 ± 16.4	57 ± 7.05	148 ± 18.8	ND

[1]GS = grass silage; CS = corn silage; FWCW = fermented whole crop wheat; UPWCW = urea processed whole crop wheat.
[2]ND = not determined.

Table 1.2. Effect of forage source on daily DMI (kg/d) by steers[1]

Item	GS	CS	FWCW	UPWCW	SEM	*P*-value
Forage	5.36[a]	8.06[b]	7.71[b]	7.93[b]	0.59	0.04
Concentrate	2.26	2.26	2.26	2.26	-	
Total	7.62[a]	10.32[b]	9.97[b]	10.19[b]	0.59	0.04

[a-b]Within a row, means without a common superscript letter differ (P<0.05).
[1]GS = grass silage; CS = corn silage; FWCW = fermented whole crop wheat; UPWCW = urea processed whole crop wheat.

Table 1.3. Effect of forage source on pH, concentrations of ammonia, lactic acid and total VFA and molar proportions of individual VFA in the rumen of steers[1]

Item	GS	CS	FWCW	UPWCW	SEM	*P*-value
pH	6.57	6.31	6.43	6.45	0.052	0.07
D-Lactate, mg/L	50	69	23	26	19.2	0.15
L-Lactate, mg/L	36	51	14	12	15.3	0.11
VFA, m*M*	62[a]	76[b]	67[ab]	64[a]	3.9	0.03
NH_3, mg/L	103[a]	28[b]	88[a]	160[c]	11.7	<0.001
Molar proportions, mmol/mol VFA						
Acetic acid	672[a]	621[b]	650[a]	674[a]	10.3	0.003
Propionic acid	182[a]	194[a]	174[ab]	153[b]	9.2	0.04
Butyric acid	117[a]	147[c]	139[b]	140[b]	2.5	<0.001
Valeric acid	29	38	38	34	3.0	0.05
Acetate:propionate ratio	3.7[a]	3.3[a]	3.8[a]	4.4[b]	0.16	0.04

[a-c]Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]GS = grass silage; CS = corn silage; FWCW = fermented whole crop wheat; UPWCW = urea processed whole crop wheat.

Table 1.4. Effect of forage source on dietary intake, nutrient flow to the omasum and apparent nutrient digestibility in steers[1]

Item	GS	CS	FWCW	UPWCW	SEM	*P*-value
OM						
Intake, kg/d	6.98[a]	9.89[b]	9.42[b]	9.68[b]	0.55	0.006
OM flow, kg/d	2.12[a]	4.37[b]	4.95[b]	5.33[b]	0.68	0.01
Ruminal digestibility						
Apparent	0.694[a]	0.559[b]	0.491[b]	0.456[b]	0.050	0.02
True	0.828[a]	0.702[ab]	0.661[b]	0.577[b]	0.059	0.03
Microbial OM flow, kg/d	0.95	1.42	1.72	1.17	0.22	0.05
Total tract digestibility	0.802[a]	0.789[a]	0.720[b]	0.756[ab]	0.022	0.03
NDF						
Intake, kg/d	3.57	3.41	3.55	3.71	0.29	0.78
NDF flow, kg/d	1.00[a]	1.66[ab]	2.23[b]	2.13[b]	0.27	0.02
Ruminal digestibility	0.716[a]	0.517[b]	0.379[b]	0.428[b]	0.053	0.005
Digested in rumen, kg/d	2.57[a]	1.76[b]	1.32[b]	1.59[b]	0.24	0.009
Total tract digestibility	0.770[a]	0.600[b]	0.470[c]	0.622[b]	0.045	0.002
Ruminal/total tract digestibility	0.932[a]	0.866[a]	0.820[a]	0.689[b]	0.051	0.03
Starch						
Intake, kg/d	0.846[a]	3.32[b]	2.74[b]	3.03[b]	0.129	<0.001
Starch flow, kg/d	0.147	0.539	0.576	0.668	0.2367	0.23
Ruminal digestibility	0.825	0.837	0.798	0.791	0.0665	0.88
Digested in rumen, kg/d	0.87[a]	2.99[b]	2.30[c]	2.44[bc]	0.228	0.004
Total tract digestibility	0.972[a]	0.986[b]	0.980[c]	0.983[bc]	0.0025	0.004
Ruminal/total tract digestibility	0.848	0.848	0.810	0.804	0.0673	0.86

[a-c]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]GS = grass silage; CS = corn silage; FWCW = fermented whole crop wheat; UPWCW = urea processed whole crop wheat.

Table 1.5. Effect of forage source on N intake, omasal N flow, microbial efficiency, and N digestion in steers[1]

Item[2]	GS	CS	FWCW	UPWCW	SEM	*P*-value
N intake, g/d	170[a]	182[ab]	213[b]	276[c]	12.8	<0.001
N flow, g/d	151[a]	263[b]	269[b]	307[b]	29.8	0.009
NAN flow, g/d	149[a]	258[b]	263[b]	295[b]	29.2	0.01
N flow/N intake	0.88[a]	1.44[b]	1.26[bc]	1.10[ac]	0.12	0.02
Microbial N flow, g/d	78[a]	126[bc]	146[c]	101[ab]	15.9	0.02
NANMN, g/d	71[a]	132[ab]	117[a]	194[b]	28.8	0.02
Microbial efficiency						
g of N/kg OMAD	16.2[a]	20.8[a]	32.5[b]	23.9[ab]	3.6	0.02
g of N/kg OMTD	13.5[a]	16.6[a]	23.3[b]	18.5[ab]	2.1	0.02
Digestibility						
Apparent ruminal	0.12[a]	-0.44[b]	-0.24[bc]	-0.11[ac]	0.11	0.02
True ruminal[7]	0.57	0.25	0.42	0.26	0.12	0.11
Total tract	0.732	0.633	0.662	0.661	0.031	0.08

[a-c]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]GS = grass silage; CS = corn silage; FWCW = fermented whole crop wheat; UPWCW = urea processed whole crop wheat.

[2]NAN = non-ammonia nitrogen; NANMN = non-ammonia non-microbial nitrogen; OMAD = OM apparently digested in the rumen; OMTD = OM truly digested in the rumen

Table 1.6. Effect of forage source on plasma concentrations of glucose, β-hydroxybutyrate, and urea in steers[1]

Item	GS	CS	FWCW	UPWCW	SEM	*P*-value
Glucose, m*M*	4.13	4.25	4.26	4.27	0.10	0.53
Beta hydroxybutyrate, m*M*	0.33[a]	0.51[c]	0.43[b]	0.46[b]	0.015	<0.001
Urea, m*M*	3.97[a]	2.43[b]	3.63[a]	5.82[c]	0.22	<0.001

[a-c]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]GS = grass silage; CS = corn silage; FWCW = fermented whole crop wheat; UPWCW = urea processed whole crop wheat.

EXPERIMENT 2: Intake, rumen fermentation and nutrient flow to the omasum in beef cattle fed grass silage fortified with sucrose and/or supplemented with concentrate

1. Introduction

Grass silage makes an important contribution to beef cattle diets in Ireland and many parts of Northern Europe. It is generally characterised by a low concentration of water soluble carbohydrates (WSC) and a high proportion of soluble non-protein N (NPN) (McDonald et al., 1991). The former are low due to their fermentation during ensilage to products that make a relatively small direct energy contribution to rumen fermentation. As the rate of degradation of the N products of ensilage in the rumen is high (Givens and Rulquin, 2004), these characteristics can result in a potential energy imbalance within the rumen and lead to inefficient capture of silage N by rumen micro-organisms, low yields of microbial crude protein (CP) and an increased loss of N to the environment (Dewhurst et al., 2000). This excretion of N to the environment is undesirable, particularly with the progressive constraints of environmental legislation in many parts of the world.

Synchronizing the supply of energy and N release in the rumen has been suggested to improve the efficiency of microbial growth and animal performance (Hoover and Stokes, 1991) although evidence is limited (Huhtanen and Shingfield, 2005). Research has shown that carbohydrate, sugars in particular, supplementation of grass silage can improve efficiency of microbial CP synthesis (Givens and Rulquin, 2004). For this reason there has been increased interest in selecting grass cultivars with elevated concentrations of WSC, and of subjecting them to ensilage technologies that restrict WSC catabolism. Grasses of high WSC concentration had a better efficiency of N use in dairy cows (Miller et al., 2001), higher live-weight gain in lambs (Lee et al., 2001) and beef cattle (Marley et al., 2005) and higher dry matter (DM) intake in beef cattle (Lee et al., 2002) *versus* lower WSC grasses. The principle underlying the benefits of elevated WSC in grass on ruminal N use efficiency should apply equally, or more so, to silage where the ratio of WSC to soluble NPN is generally more unfavourable (Davies et al., 2005). In practical farming, grass silage is generally not offered alone to beef cattle, and concentrate supplementation is used to overcome deficiencies in nutrient supply (McGee, 2005). The latter can also positively impact on efficiency of N use (Jaakkola and Huhtanen, 1993) and animal performance (McGee, 2005). Therefore, the potential interaction between silage WSC and concentrate supplementation needs to be quantified. While a number of studies have examined effects of adding rapidly fermented carbohydrates to grass silage-based diets in the form of molasses (Huhtanen, 1988; Moloney et al., 1994) or sucrose (Huhtanen, 1987; Khalili and Huhtanen, 1991a; 1991b; Chamberlain et al., 1993; Oh et al., 1999), delivery method for the carbohydrate was usually as a distinct supplement or by continual infusion rather than a uniform inclusion in the forage. Furthermore, there are few data quantifying flow of nutrients from the rumen of beef cattle consuming these types of diets.

The underlying hypothesis of the current study was that mixing sucrose with unwilted (i.e., extensively fermented) grass silage would improve efficiency of rumen metabolism and enhance nutrient supply to the omasum. The objective was to quantify the interaction between effects of elevating silage WSC concentration, as potentially enabled by grass cultivar, and effects of supplementation with a level of a starch-based concentrate commonly used on many beef farms, on feed intake, rumen fermentation and nutrient supply to the omasum in beef cattle.

2. Materials and Methods

All procedures in the study were conducted under experimental licence from the Irish Department of Health and Children in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulation, 1994.

2.1 Animals and diets

The experiment used four ruminally fistulated Holstein Friesian steers (580 ± 16.3 kg) in four 21 day periods × four diets in a Latin square design experiment with a 2 × 2 factorial arrangement of treatments. Each period consisted of a 9 day dietary adaptation followed by 12 days for sample collection. The four experimental diets offered were grass silage only (G), grass silage plus 3 kg concentrates/day (GC), grass silage plus 90 g sucrose/kg DM (GS) and grass silage plus 90 g sucrose/kg DM plus 3 kg concentrates/day (GCS). Treatments were selected from the range examined in the concurrent beef production study of O'Kiely et al. (2005). Grass silage was a 1st cut silage harvested from a perennial ryegrass sward (*Lolium perenne* L., cv. Fennema) in early June 2003, with a precision-chop harvester and ensiled, without wilting, in roofed horizontal silos. The herbage was rolled thoroughly, to ensure expulsion of air, before covering beneath 2 layers of black polythene sheeting

and fully covered with tyres. Cores were taken from the silage to establish DM content and sucrose was added throughout the experiment at an appropriate rate (g/kg DM) based on these values. For the sucrose-containing diets, sucrose was mixed with fresh silage each morning for 10 min using a complete diet feeder (Abbey feeder wagon, Abbey Farm Machinery, Nenagh, Co. Tipperary, Ireland). Likewise, silage for the non-sucrose treatments was similarly processed. Sucrose was from Irish Sugar (Greencore, Ireland) and was domestic consumption grade. The cereal-based concentrate consisted of 800 g rolled barley grain, 130 g extracted soya-bean meal, 50 g liquid molasses and 20 g minerals and vitamins per kg, and was offered on top of the silage. Forages were offered *ad libitum* in two equal daily feedings at 0830 and 2030 h until day 9 of each period and then similarly at 0.90 of *ad libitum* for the remaining 12 days. Intake was restricted to minimize between and within day variations in intake during intensive sampling (Ahvenjärvi et al., 2000). The 3.0 kg of concentrate were offered twice daily at 0830 and 2030 h in two equal meals until day 9 and then similarly at 0.90 of their allocation until the end of each period.

2.2 Feedstuff sampling

Forages and concentrates offered were sampled daily in duplicate during the collection period. Forage refusals, if any, were weighed and sampled daily in duplicate during days 9 to 21. All samples were stored at -18^{o}C. Forage samples were pooled for days 9-12 and at 2-day intervals from day 13 to give 5 samples per period. As the chemical composition of a cereal-based concentrate is more uniform, concentrate samples were pooled for days 9-12 and 13-21.

2.3 Blood sampling and analyses

On day 10, two blood samples were obtained by jugular venipuncture from each steer immediately before and 3 and 6 h after the morning feeding using separate vacutainers containing lithium heparin and sodium fluoride-EDTA K3 as anticoagulants. Blood samples were then centrifuged at 2500 × *g* for 20 min at 4^{o}C. The plasma was stored at -18^{o}C prior to chemical analysis.

2.4 Rumen fluid sampling

Rumen fluid samples of approximately 200 ml were collected through the rumen cannulae (Moloney and Flynn, 1992) immediately before feeding at 0830 and then at 1000, 1130, 1300, 1430, 1600, 1730 and 1900 h on day 11 of each period to assess rumen fermentation characteristics. Rumen fluid pH was measured immediately after collection using an Orion digital pH meter (Orion, SA720; Thermo Fisher Scientific, Waltham, MA, USA) and glass electrode. A 20 ml sub-sample was acidified with 0.5 ml of 9M sulphuric acid and stored at –18 ^{o}C for subsequent analysis.

2.5 Indigestible marker administration

The markers used to estimate digesta flows in the cannulated steers were indigestible NDF (INDF) for the large particle phase (Huhtanen et al., 1994), Yb-acetate for the small particle phase (Siddons et al., 1985) and Co-EDTA for the fluid phase (Udén et al., 1980). Following a priming dose of 2.5 g of Yb-acetate and 8.0 g Co-EDTA, the same amounts of Yb-acetate and Co-EDTA per day were dissolved in 4 l of distilled water and were continuously infused into the rumen using a multi-channel peristaltic pump (Watson Marlow, 502 S, Falmouth, UK) from day 11 to 20.

2.6 Digesta sampling

To determine digesta flow, 400 ml spot samples from the omasal canal were collected using an alternating pressure - vacuum system as described by Huhtanen et al. (1997) and modified by Ahvenjärvi et al. (2000, 2001). Samples were collected twice daily at an interval of 6 h for days 16-19 and started at 0830 hours on day 16 and advanced 90 min each day such that samples represented each hour between the morning and evening feeding; considered to be representative of the entire feeding cycle (Ahvenjärvi et al., 2006). These samples were collected on ice and were then stored at - 18^{o}C. True digesta flows into the omasum were calculated according to the triple marker method (France and Siddons, 1986) whereby the digesta was separated into three phases, large and small particle phase and liquid phase, from which true digesta was reconstituted (Ahvenjärvi et al., 2000). Reticulorumen fluid samples (500 ml) were obtained manually once daily according to the method of Ahvenjärvi et al. (2002) at 1730, 1430, 0830 and 1130 on days 16, 17, 18, and 19, respectively, for microbial CP determination. Care was taken to avoid mixing of ruminal and reticular contents during sampling. The microbial pellet was prepared according Ahvenjärvi et al. (2000).

2.7 Faecal sampling

Faecal grab samples (~500 g) were obtained via rectal palpation at the same times as omasal samples (Ahvenjärvi et al., 2001) and stored at -18^{o}C. At the end of the sampling period, these samples were thawed and pooled on an equal volume basis per steer and period.

2.8 Sucrose loss post mixing

A separate experiment was completed to examine effects of silage respiration on residual sucrose concentrations following addition of sucrose. The silage used differed from that used in the main experiment and of which six sub-samples were collected that were fresh (Fresh) or that were stored at 20°C for 3 days (Respire) and mixed with 0, 60, 90 or 120 g sucrose/kg DM. Three of the six samples from each of the above eight treatments were immediately placed in a forced air circulation oven at 40°C for 48 h (0) and the remainder were stored at 20°C for a further 24 h and were then placed in the oven (24).

2.9 Chemical analysis

The DM content of the forages and concentrates was determined after drying at 85°C for 24 h and 16 h, respectively, in an oven with forced air circulation. Forage DM content was corrected for volatile losses according to Porter and Murray (2001). Composited forage and concentrate samples were sub-sampled and one sub-sample was subsequently oven dried at 40°C for 48 h and then ground through a Wiley mill (1 mm screen) for analysis of *in vitro* DMD (Tilley and Terry, 1963), neutral detergent fibre of grass silage (NDFom) and concentrates (aNDFom) and acid detergent fibre (ADFom) (Van Soest et al., 1991), ash by burning in a muffle furnace at 550°C for 5 h, N using a Dumas-type N analyser (Leco FP-428; Leco Corporation, St Joseph, MI, USA), water soluble carbohydrates (WSC) (anthrone method; Thomas, 1977), starch (McCleary et al., 1997) and acid insoluble ash (AIA) (Van Keulen and Young, 1977). One composited sample of faeces per steer was dried at 98°C for 48 h for DM determination while another was dried at 60°C for 48 h and milled for the analysis of Yb, Co, INDF, ash, N, aNDFom and AIA. Omasal digesta was freeze dried and ground prior to analysis of Yb, Co, INDF, ash, N and aNDFom.

Rumen fluid and silage juice samples were analysed for ammonia N measured using the Olympus AU400 and the Thermo Electron Infinity Ammonia Liquid Stable Reagent kinetic method) and lactic acid using the Olympus AU400 and the L-Lactic Acid UV-method test kit (Boehringer Mannheim / R-Biopharm catalogue number 10139084035) with the enzyme D-Lactate Dehydrogenase (Boehringer Mannheim / R-Biopharm catalogue number 10106941001). Concentrations of volatile fatty acids (VFA) were measured using an automated gas chromatograph (Shimadzu Gas Chromatography GC-8A; Shimadzu Corporation, Kyoto 604-8511, Japan) (Ranfft, 1973).

Omasal digesta and faeces were analysed for Yb and Co according to Hart and Polan (1984). The procedure to determine INDF concentration of feeds, faeces and digesta was as described by Ahvenjarvi et al. (2000). Purine bases in the microbial pellet were determined according to the method of Zinn and Owens (1986) and modifications of Makkar and Becker (1999).

Plasma urea (Kinetic urease method, Olympus catalogue number OSR6134), β-hydroxybutyrate (Randox Laboratories catalogue number RB1005) and glucose (Hexokinase method, Olympus catalogue number OSR6121) were analysed using an Olympus AU 400 Clinical Analyser.

2.10 Statistical analyses

Feed intake, rumen fermentation, nutrient flows and blood parameters were analysed as a 2 × 2 factorial arrangement of treatments using the general linear procedure in PROC GLM of SAS (2003) appropriate for a Latin Square design. The statistical model included terms for animal, period, sucrose, concentrate and the sucrose × concentrate interactions.

3. Results

3.1 Feed composition

The silage used had low *in vitro* DMD and CP values (Table 2.1). However, it was well preserved as shown by its high proportion of lactic acid and its low pH and moderate ammonia-N level. Addition of sucrose at a rate of 90 g/kg silage DM increased the WSC content of the silage by an additional 36 g WSC /kg silage DM.

3.2 Sucrose loss post mixing

Large respiration losses occured when sucrose was mixed with heated silage (Figure 2.1) and then allowed to respire for a further 24 h. Mixing 90 g sucrose/kg DM with the Respire 24 treatment only increased the WSC concentration by 34 g/kg DM.

3.3 Feed intake

Supplementation of grass silage with concentrate decreased ($P<0.01$) silage DM intake and increased ($P<0.001$) total DM intake (Table 2.2). Addition of sucrose to grass silage had no effect on silage or total DM intake.

There was no sucrose × concentrate (S × C) interaction. Concentrates and sucrose comprised 0.29 and 0.07, respectively, of total DM intake.

3.4 Rumen fermentation

There was an S × C interaction ($P<0.05$) for rumen pH whereby addition of sucrose to grass silage alone decreased pH and to grass silage plus concentrate had no effect (Figure 2.2 and Table 2.3). Concentrate supplementation increased rumen concentrations of ammonia ($P<0.01$), D-lactate ($P<0.05$) and total VFA ($P<0.05$), and the molar proportions of butyric ($P<0.001$) and valeric ($P<0.05$) acids. There was a tendency ($P<0.10$) for concentrate supplementation to decrease the proportion of propionate. There was no effect of addition of sucrose on the molar proportions of VFA.

3.5 OM and NDF omasal flow and digestibility

Concentrate supplementation increased ($P<0.001$) organic matter (OM) intake, OM and microbial OM flow ($P<0.05$), the quantities of OM apparently (OMAD) and truly digested (OMTD) in the rumen, and total tract OM digestibility ($P<0.01$) (Table 2.4). There was a tendency ($P<0.10$) for addition of sucrose to increase the quantity of OMTD in the rumen and true ruminal OM digestibility. True ruminal OM digestibility was decreased ($P<0.05$) by concentrate supplementation. There was an S × C interaction ($P<0.01$) for omasal flow and apparent ruminal digestibility of OM, whereby fortification of grass silage with sucrose had no effect on omasal OM flow or ruminal digestibility, while the addition of sucrose to grass silage plus concentrate decreased omasal OM flow but increased ruminal digestibility of OM. Sucrose supplementation had no effect on aNDFom intake, omasal flow or ruminal aNDFom digestibility whereas concentrate supplementation increased ($P<0.05$) aNDFom omasal flow and decreased ($P<0.05$) ruminal aNDFom digestibility. There was no effect of either sucrose or concentrate supplementation on total tract aNDFom digestibility or ruminal digestibility as a proportion of total tract digestibility.

3.6 Nitrogen metabolism

Concentrate supplementation increased ($P<0.001$) N intake and N, NAN and non-ammonia non-microbial N (NANMN) ($P<0.01$) flow to the omasum (Table 2.5). Addition of sucrose decreased ($P<0.05$) N intake and increased ($P<0.01$) N flow as a proportion of N intake. A S × C interaction ($P<0.05$) for N flow as a proportion of N intake showed that addition of sucrose to grass silage increased ($P<0.05$) the proportion whereas addition of sucrose to grass silage plus concentrates had no effect. Microbial CP synthesis increased ($P<0.05$) with concentrate supplementation while sucrose had no effect. There was no effect of either concentrate or sucrose on the efficiency of microbial CP synthesis, or on the efficiency of converting feed N into MN. There was a S × C interaction ($P<0.05$) for apparent ruminal N digestibility whereby addition of sucrose to grass silage decreased it, while addition of sucrose to grass silage plus concentrates had no effect. Sucrose supplementation had no effect on true ruminal or total tract digestibility of N whereas concentrate supplementation decreased ($P<0.01$) true ruminal N digestibility and increased ($P<0.01$) total tract N digestibility.

3.7 Blood metabolites

Blood metabolite data are presented as the mean of 3 samples collected throughout the day (Table 2.6). There was no effect of either sucrose or concentrate supplementation on the mean glucose or plasma urea levels. Concentrate supplementation increased ($P<0.001$) plasma β-hydroxybutyrate levels, while fortification with sucrose had no effect. There was no S × C interactions ($P>0.05$) for any blood metabolites.

4. Conclusions

Results suggest that an increase in grass silage WSC of a magnitude that could be achieved by plant breeding over a one decade duration would have relatively little effect on intake, rumen fermentation, MN flow, efficiency of microbial CP synthesis or plasma metabolites of beef cattle compared to effects of supplementing silage with 3 kg of a starch-based concentrate per day.

Table 2.1: Chemical composition of silages and concentrate (g/kg DM unless otherwise stated, and not pH) (s.d. in parentheses)

	Grass silage	Grass silage + 90g sucrose/kg DM	Concentrate
[a]Dry matter (g/kg)	197 (15.9)	206 (15.1)	840 (0.6)
in-vitro DMD (g/kg)	661 (25.4)	677 (24.4)	876 (12.0)
in-vitro DOMD	595 (27.5)	617 (31.4)	829 (6.7)
Ash	87 (11.2)	83 (18.6)	48 (5.4)
Crude protein	110 (6.8)	104 (7.7)	146 (23.3)
NDFom	595 (22.7)	582 (19.6)	nd
aNDFom	nd[b]	nd	117 (20.7)
ADF	375 (14.3)	354 (11.5)	53 (4.2)
WSC	11(5.1)	47 (13.8)	74 (8.1)
Starch	nd	nd	455 (34.9)
pH	3.83 (0.10)	3.96 (0.19)	nd
Lactic acid	98 (23.7)	84 (21.4)	nd
Acetic acid	24.5 (4.8)	24.1 (4.7)	nd
Propionic acid	1.8 (0.7)	2.0 (0.7)	nd
Butyric acid	3.1 (2.0)	4.7 (3.0)	nd
Ethanol	21.4 (5.9)	19.1 (5.7)	nd
Ammonia N (g/kg N)	118 (15.9)	118 (25.4)	nd

[a] Dry matter corrected for loss of volatiles during oven drying
[b]Not determined

Table 2.2: Mean grass silage, concentrate and total DM intakes (kg/d) of steers fed 0.9 of *ad-libitum* grass silage only (G), grass silage plus 3 kg concentrates/day (GC), grass silage plus 90 g sucrose/kg DM (GS) and grass silage plus 90 g sucrose/kg DM plus 3 kg concentrates/day (GCS)

	Treatment					Significance[a]		
	G	GC	GS	GCS	S.E.M.	Sucrose (S)	Concentrate (C)	S × C
Silage	6.03	5.72	6.25	5.65	0.091	NS	**	NS
Concentrate		2.28		2.28				
Total	6.03	7.99	6.25	7.93	0.092	NS	***	NS

NS: non-significant, ** P<0.01, ***P<0.001
[a] Significance of main effects; S = (G + GC) v (GS + GCS), C = (G + GS) v (GC + GCS), S × C = (interaction between main effects)

Table 2.3: Rumen fermentation characteristics of steers fed 0.9 of *ad-libitum* grass silage only (G), grass silage plus 3 kg concentrates/day (GC), grass silage plus 90 g sucrose/kg DM (GS) and grass silage plus 90 g sucrose/kg DM plus 3 kg concentrates/day (GCS)

	Treatment					Significance[a]		
	G	GC	GS	GCS	S.E.M.	Sucrose (S)	Conc. (C)	S x C[b]
pH	6.83[a]	6.64[b]	6.72[b]	6.65[b]	0.023	NS	**	*
D Lactate (mg/l)	16	28	13	31	5.2	NS	*	NS
L Lactate (mg/l)	20	32	15	29	6.3	NS	NS	NS
Total VFA (mmol/l)	73	84	79	90	3.2	NS	*	NS
Ammonia (mg/l)	58.8	81.3	53.5	70.9	4.61	NS	**	NS
Molar proportions (mmol/mol)								
Acetic acid	686	675	682	674	7.2	NS	NS	NS
Propionic acid	196	184	194	184	5.6	NS	†	NS
Butyric acid	85	109	89	115	3.1	NS	***	NS
Valeric acid	34	32	35	28	1.4	NS	*	NS
Acetate:Propionate ratio	3.5	3.8	3.6	3.8	0.15	NS	NS	NS

NS: non-significant, * P<0.05, ** P<0.01, ***P<0.001, †P<0.10.
[a] Significance of main effects; S = (G + GC) v (GS + GCS), C = (G + GS) v (GC + GCS), S × C = (interaction between main effects)
[b] Within row, means with the same superscripts do not significantly differ

Table 2.4: Dietary intake, nutrient flow to the omasum and apparent nutrient digestibility in steers fed 0.9 of ad-libitum grass silage only (G), grass silage plus 3 kg concentrates/day (GC), grass silage plus 90 g sucrose/kg DM (GS) and grass silage plus 90 g sucrose/kg DM plus 3 kg concentrates/day (GCS)

	Treatment					Significance[a]		
	G	GC	GS	GCS	S.E.M.	Sucrose (S)	Conc. (C)	S x C[b]
OM								
Intake (kg/d)	5.51	7.39	5.73	7.35	0.084	NS	***	NS
OM flow	1.97[a]	3.02[b]	2.14[a]	2.69[c]	0.051	NS	***	**
OMAD[c]	0.642[a]	0.590[b]	0.627[a]	0.636[a]	0.0073	†	*	**
OMAD (kg/d)	3.53	4.37	3.59	4.66	0.0948	NS	***	NS
Microbial OM flow	1.02	1.43	1.26	1.41	0.085	NS	*	NS
OMTD[d]	0.829	0.783	0.848	0.825	0.0133	†	*	NS
OMTD (kg/d)	4.55	5.80	4.85	6.06	0.127	†	***	NS
Total tract digestibility	0.675	0.728	0.664	0.742	0.0175	NS	**	NS
aNDFom								
Intake (kg/d)	3.59	3.67	3.64	3.55	0.053	NS	NS	NS
aNDFom flow	1.22	1.48	1.23	1.30	0.051	NS	*	NS
Ruminal digestibility	0.661	0.594	0.665	0.634	0.0159	NS	*	NS
Total tract digestibility	0.671	0.633	0.650	0.658	0.0221	NS	NS	NS
Ruminal/total digestibility	0.985	0.950	1.033	0.980	0.0427	NS	NS	NS

NS: non-significant, * P<0.05, ** P<0.01, ***P<0.001, †P<0.10.
[a] Significance of main effects; S = (G + GC) v (GS + GCS), C = (G + GS) v (GC + GCS), S × C = (interaction between main effects)
[b] Within row, means with the same superscripts do not significantly differ
[c] OM apparently digested in the rumen (OM intake – OM flow)
[d] OM truly digested in the rumen (OMAD + microbial OM flow), assuming the ash content of microbial DM was 0.10.

Table 2.5: Nitrogen intake, N, NAN, microbial N and NANMN flow entering the omasum (g/d) and apparent nutrient digestibility in steers fed 0.9 of *ad-libitum* grass silage only (G), grass silage plus 3 kg concentrates/day (GC), grass silage plus 90 g sucrose/kg DM (GS) and grass silage plus 90 g sucrose/kg DM plus 3 kg concentrates/day (GCS)

	Treatment					Significance[a]		
	G	GC	GS	GCS	S.E.M.	Sucrose (S)	Conc. (C)	S × C[b]
N intake (g/d)	106	153	104	147	1.6	*	***	NS
N flow (g/d)	90	165	115	159	6.7	NS	***	NS
NAN flow (g/d)	87	157	113	153	6.3	NS	***	NS
N flow/N intake	0.84[a]	1.08[b]	1.11[b]	1.08[b]	0.053	*	NS	*
Microbial N flow (g/d)	80	112	99	105	7.1	NS	*	NS
NANMN (g/d)	7	45	14	48	7.1	NS	**	NS
N use efficiency, g of MN/g of feed N	0.76	0.73	0.95	0.71	0.058	NS	NS	NS
Microbial efficiency								
g of MN/kg OMAD	23.0	26.1	27.9	22.6	1.85	NS	NS	NS
g of MN/kg OMTD	17.7	19.3	20.4	17.3	1.10	NS	NS	NS
N digestibility								
Apparent ruminal	0.159[a]	-0.077[b]	-0.114[b]	-0.080[b]	0.0535	*	NS	*
True ruminal[c]	0.920	0.651	0.837	0.635	0.0561	NS	**	NS
Total tract	0.533	0.635	0.455	0.616	0.0354	NS	**	NS

NS: non-significant, * P<0.05, ** P<0.01, ***P<0.001

[a] Significance of main effects; S = (G + GC) v (GS + GCS), C = (G + GS) v (GC + GCS), S × C = (interaction between main effects)

[b] Within row, means with the same superscripts do not significantly differ

[c] True ruminal N digestibility: 1 – [(N flow – microbial N)/N intake]

Table 2.6: Plasma metabolites in steers fed 0.9 of *ad-libitum* grass silage only (G), grass silage plus 3 kg concentrates/day (GC), grass silage plus 90 g sucrose/kg DM (GS) and grass silage plus 90 g sucrose/kg DM plus 3 kg concentrates/day (GCS)

	Treatment					Significance[a]		
	G	GC	GS	GCS	S.E.M.	Sucrose (S)	Conc. (C)	S x C
β hydroxybutyrate (mmol/l)	0.24	0.34	0.26	0.34	0.013	NS	***	NS
Glucose (mmol/l)	3.73	3.91	3.79	4.04	0.117	NS	NS	NS
Urea (mmol/l)	2.08	2.53	1.87	2.33	0.258	NS	NS	NS

NS: non-significant, ***P<0.001

[a] Significance of main effects; S = (G + GC) v (GS + GCS), C = (G + GS) v (GC + GCS), S × C = (interaction between main effects)

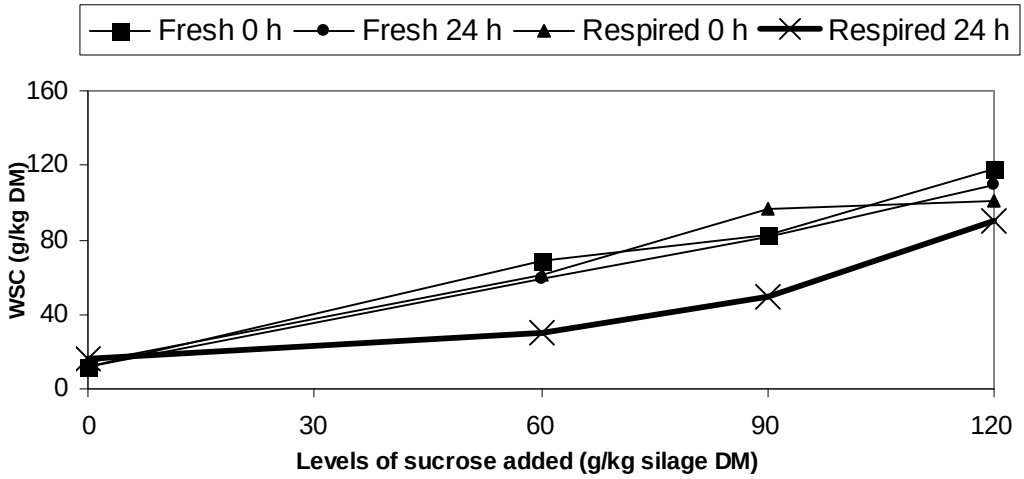

Fig. 2.1. Concentration of WSC in fresh and respired grass silage mixed with 0, 60, 90 and 120 g sucrose/kg silage DM and dried immediately (0) or following 24 h (24) at 20^{o}C (S.E.M. = 6.6)

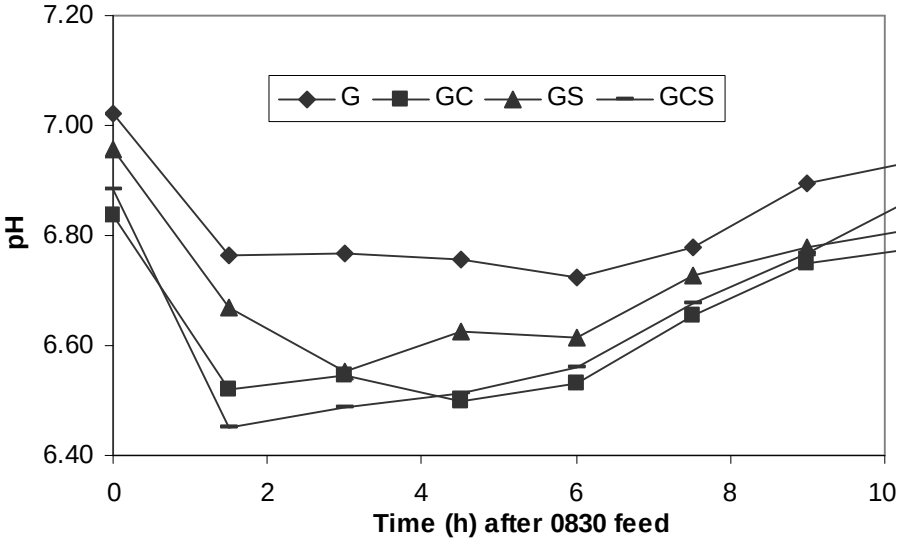

Fig. 2.2. Effects of concentrate supplementation and addition of sucrose to grass silage on rumen fluid pH in steers fed 0.9 of *ad-libitum* grass silage only (G), grass silage plus 3 kg concentrates/day (GC), grass silage plus 90 g sucrose/kg DM/day (GS) and grass silage plus 90 g sucrose/kg DM plus 3 kg concentrates/day (GCS) (S.E.M. = 0.043)

EXPERIMENT 3: Effect of grass regrowth interval on intake, rumen digestion and nutrient flow to the omasum in beef cattle

1. Introduction

In many parts of Northern and Western Europe, current climatic conditions dictate that grass is the principal forage for beef production. Production and efficient utilisation of high yields of high nutritive value grass throughout an extended grazing season is critical for cost efficient beef production (O'Riordan et al., 2000).

Due to environmental legislation and (or) stipulations in environmental schemes such as the European Union (EU) Rural Environmental Protection Scheme many farmers will now need to operate to lower input criteria than heretofore. The emphasis will be to restrict the application of nitrogen (N) fertilisers and, consequently, many grassland management systems will reduce in intensity.

As livestock production has been identified as a major source of N loss to the environment, it is important to reduce N excretions by improving N utilisation (Hoekstra et al., 2007). For the grazing ruminant this inefficiency of N capture is largely due to the high crude protein (CP) concentrations and imbalances in the supply of carbohydrate and protein in the rumen (Hoekstra et al., 2007). Nitrogen release into the environment by grazing animals can be reduced by decreasing the crude protein content of the herbage by lowering N fertilisation or by feeding more mature grass (Peyraud and Astigarraga, 1998; Hoekstra et al., 2007). Furthermore, O'Riordan (1997) and (1998) showed that allowing grass to grow for an additional 7 days (d) (21 vs. 28) resulted in extra herbage production, which was equivalent to the production response to approximately 150 kg fertiliser N/ha.

However, as grass matures, the nutritive quality generally declines, due to increased lignification, a decreased ratio of leaves to stem and a decrease in CP concentration (Delagarde et al., 2000). Under average grass growing conditions in Ireland, regrowth intervals (RI) of not greater than 24 to 26 d are recommended for spring grazing of beef cattle, as exceeding this can lead to poorer pasture utilisation and swards of lower quality later in the season (O'Riordan et al., 2000).

Quantitative knowledge on rates of passage of nutrients out of the rumen is rather limited compared with that on degradation rates (Dijkstra et al., 2007). Whereas a number of studies have examined the effects of grass maturity or RI on rumen digestion and nutrient flow of grass silage (Bosch et al., 1992; McAllan et al., 1994; Rinne et al., 1997; 2002; DeVisser et al., 1998; Cone et al., 1999), there is a lack of similar data pertaining to the effects of fresh grass. Furthermore, studies comparing effects of grass RI on rumen fermentation and digestion (Mambrini and Peyraud, 1994; Ribeiro Filho et al., 2003) have generally used much longer RI than is practical for grazing management on temperate pastures, and thus may not be indicative of what is commercially feasible.

The objective of the current study was to examine the effects of increasing grass RI from 28 to 38 d on intake, rumen fermentation and digestion, and nutrient supply to the omasum in beef cattle.

2. Materials and Methods

In order to provide an adequate herbage allowance and utilisation rate for beef cattle in rotationally grazed grassland systems, recommended RI are generally between 24 and 26 days during the spring (O'Riordan et al., 2000). This study was designed to quantify the passage of nutrients out of the rumen using contrasting RI that could be integrated into a practical rotationally grazed system. Originally the treatments were to consist of a 20 and 30 d RI, but due to a delay in grass growth at the beginning of the experiment, the RI had to be extended by a further 8 d to achieve a pre-grazing herbage mass sufficient to meet the estimated intake capacity of the experimental animals.

2.1 Experimental area

The study was carried out Teagasc, Grange Beef Research Centre (Longitude 6^{o} 40' W; Latitude 53^{o} 30' N; Elevation 92 m above sea level) between 4^{th} April and 12^{th} June 2005. The soil type was a moderately well drained Brown Earth of medium to high base content and of clay loam texture (Gardiner, 1962). Meteorological data were recorded (O'Rielly, 1986) at Grange Beef Research Centre. The 20-year (1982-2002) mean annual rainfall, duration of sunshine hours and daily ground temperature were 849 mm, 1230 h and 9.1^{o}C, respectively. The 5-year (2001-2005) mean annual grass production at the centre determined according to O'Riordan (1997) was 10,576 kg DM/ha.

The experimental area (2 ha) consisted of a predominantly perennial ryegrass (*Lolium perenne cv. Melle)* sward sown in 2002. It received 50 kg fertiliser N in the form of urea on 7 February and an application of 25 and 50 kg/ha of P and K, respectively, on 9 March. It was not grazed or mown prior to the start of the experiment. Treatments consisted of a 28 and 38 d RI. The area was longitudinally divided into two plots, one for each RI

and each plot was divided into 13 individual 3-d subplots. It was designed so that each subplot would provide sufficient herbage for 3 d zero-grazing during the feeding study. For preparation of the experimental herbage, 39 and 29 d prior to feeding, the herbage was harvested (commencing on 4 and 14 April for the 38 and 28 d RI, respectively), to a 6 cm stubble height from the respective subplots using an Agria mower, removed and 33 kg N/ha (urea) was applied. This sequence was repeated every 3 d to ensure the difference in RI was maintained for the 2 contrasting herbages.

2.2 Management of animals

Six ruminally fistulated (fitted with 100 mm internal diameter ruminal cannulas) Holstein-Friesian steers (average bodyweight, 436 ± 15.3 kg at the start of the experiment) were used in a 2 (treatments) × 2 (periods) cross-over design experiment. Each period consisted of 17 d with 6 d of dietary adaptation and 11 d of sampling. A relatively short dietary adaptation period has been reported for fresh grass diets (Boudon and Peyraud, 2001; Stakelum and Dillon, 2003; Taweel et al., 2005).

Animals were housed in a tie-stall barn with free access to water at all times. The grass regrowth was harvested daily at 11.00 h to 6 cm stubble height using an Agria mower and was stored without further chopping at 4^{o}C until required. The grass was offered *ad libitum* in 2 equal daily meals at 08.30 and 20.30 h at proportionately 1.1 of the previous days intake until d 5 and then subsequently offered at 0.95 of the average intake of d 3, 4 and 5 until the end of each period. Intake was restricted to minimize between and within day variations in intake during intensive sampling (Ahvenjärvi et al., 2000).

2.3 Sward measurement and grass sampling

Pre-cutting sward heights were measured daily using a rising plate meter (Jenquip, Fielding, New Zealand) and pre-cutting sward mass was determined by weighing the grass and measuring the area harvested daily to 6 cm stubble height. Duplicate samples of grass offered were obtained daily. Samples were stored at -20^{o}C until processing when samples were composited for d 6-8, 9-11, 12-14 and 15-17 using a bowl chopper (Muller, Type MKT 204 Special, Saarbrucken, Germany) yielding a total of 4 samples per treatment per period for chemical analyses. The grass dry matter (DM) allowance offered daily was estimated by rapid DM determination of 3 × 50 g representative samples per treatment using microwave drying for a period of 12 min (O'Kiely, 1997).

2.4 Rumen evacuation

Rumen evacuations for determining rumen pool size and digesta kinetics, were carried out 5 h after the morning feed on d 7 and prior to morning feeding on d 8, respectively. Rumen contents were collected into an 80 l uninsulated plastic container with a stainless steel grid 20 cm from the base to facilitate the separation of solid and liquid fractions as described by Moloney et al. (1993) with an additional modification of a tap at the base to assist the collection of liquid. Rumen liquid and solid fractions were weighed. A 500 ml sample of the liquid was obtained of which a 20 ml sub-sample was acidified with 0.5 ml 9M sulphuric acid prior to analysis of volatile fatty acids (VFA), lactic acid and ammonia. After sampling, the liquid was returned to the rumen followed by the solid fraction, which was sampled regularly during this process. The resulting solid fraction sample was thoroughly mixed, a ~2.0 kg (equivalent to 0.06 of total solid fraction) sub-sample was obtained and the remainder was returned to the rumen. The evacuating and returning of contents to the rumen lasted 45 min per animal and the rumen was empty for 2-3 min. All samples were then stored at -20^{o}C.

2.5 Rumen fluid sampling

Rumen fluid samples of approximately 200 ml to determine rumen fermentation characteristics were collected through the rumen cannulae (Moloney and Flynn, 1992) at 0830, 1030, 1230, 1430, 1630, 1830 and 2030 h on d 9 of each period to assess rumen fermentation characteristics. Rumen fluid pH was measured immediately after collection using an Orion digital pH meter (Orion, SA720) and glass electrode. A 20 ml sub-sample was acidified with 0.5 ml of 9M sulphuric acid and stored at –20 ^{o}C for subsequent analysis of VFA, lactic acid and ammonia.

2.6 Degradability in the rumen

Representative samples of grass (about 1.5 kg fresh weight) from each RI treatment were dried at 40^{o}C for 48 h in a forced air circulation oven and ground to pass a 2 mm screen. Degradability in the rumen was determined using the *in situ* nylon bag technique as outlined by Woods et al. (2002). On d 10 of each period, nylon bags (5 cm × 10 cm; 53 micron pore size, *Ankom Technology Corporation, New York*) containing 2 g of dried grass were incubated in duplicate in the rumen for 0, 2, 4, 8, 12, 24, 48 and 72 h. The undegradable neutral detergent fibre (NDF) fraction was determined separately by incubating 20 bags per treatment in the rumen for 288 h (Robinson et al., 1986). The 0 h time point represented the bags, which were not incubated in the rumen but subsequently treated in the same manner as the incubated bags. After removal from the rumen, bags were stored at -20^{o}C, until thawing, stomaching in a bicarbonate solution and machine washing. Dry matter was determined in an oven

with forced air circulation at 40°C for 48 h. Dried bags were weighed to determine DM disappearance and residues were removed and analysed to determine CP, organic matter (OM) and NDFom concentration in the DM.

2.7 Marker administration

The markers used to estimate digesta flows in the cannulated steers were indigestible NDF (INDF) for the large particle phase (Huhtanen et al., 1994), Yb-acetate for the small particle phase (Siddons et al., 1985) and Co-EDTA for the fluid phase (Uden et al., 1980). Following a priming dose of 3.75 g of Yb-acetate and 12 g LiCoEDTA, 2.5 g of Yb-acetate and 8 g of LiCoEDTA per day were dissolved in 4 L of distilled water and were continuously infused daily into the rumen using a multi-channel peristaltic pump (Watson Marlow) from d 9 to 17. The volumes of liquid infused daily were recorded.

2.8 Digesta sampling

To determine digesta flow, 400 ml spot samples from the omasal canal were collected using an alternating pressure - vacuum system as described by Huhtanen et al. (1997) and modified by Ahvenjärvi et al. (2000) and (2001) from the omasal canal twice daily at an interval of 6 h from d 14 to 17. Sampling started at 0830 hours and advanced 90 min each day to cover a 12-h period. This was considered to be representative of the entire feeding cycle. These samples were collected on ice and were then stored at - 20°C. Reticulum samples (500 ml) were obtained manually once daily according to the method of Ahvenjärvi et al. (2002) at 1730, 1430, 0830 and 1130 on d 14, 15, 16, and 17, respectively for microbial protein determination. Care was taken to avoid mixing of ruminal and reticular contents during sampling. The microbial pellet was prepared according to the method of Ahvenjärvi et al. (2000).

2.9 Faecal sampling

Faecal grab samples (~500 g) were obtained via rectal palpation at the same times as the omasal samples (Ahvenjärvi et al., 2001). These samples were stored at -20°C. At the end of the sampling period these samples were thawed and pooled on an equal weight basis, per animal per period.

2.10 Calculations

Passage and digestion kinetics from rumen evacuation data were calculated according to Robinson et al. (1987) using the following equations:

Rate of intake (k_i) = intake (kg/24 h) / rumen pool (kg)

Rate of passage (k_p) = faecal output (kg/24 h) / rumen pool (kg)

Rate of digestion (k_d) = k_i - k_p

Diet degradability data were calculated according to the first order model of Robinson et al. (1986). This model includes an insoluble potentially degradable fraction (D), which is degraded at a constant rate (k_d) with an undegradable fraction (U). The instantly degradable soluble fraction was estimated as the fraction disappearing during washing (W). The effective degradability (ED) was determined according to Salaün et al. (1999) using outflow rates which were calculated as follows: (omasal flow, kg/d)/(rumen pool size, kg) × 1/24. Protein truly digestible in the small intestine (PDI) values were calculated for both treatments according to Vérité and Peyraud (1989). The true digesta flows into the omasum were calculated according to the triple marker method (France and Siddons, 1986) whereby the digesta was separated into three phases, large and small particle phase and liquid phase, from which true digesta was reconstituted (Ahvenjärvi et al., 2000).

2.11 Chemical analysis

The DM content of the grass was determined after drying at 98°C for 16 h in an oven with forced air circulation. Pooled grass samples were freeze dried and ground through a Wiley mill (1 mm screen) for analysis of *in vitro* dry matter digestibility (DMD) (Tilley and Terry, 1963), NDFom and acid detergent fibre (ADFom) were expressed exclusive of residual ash (Van Soest et al., 1991), ash by burning in a muffle furnace at 550°C for 5 h, N using a Dumas-type N analyser (Leco FP-428; Leco Corporation, St Joseph, MI, USA), water soluble carbohydrates (WSC) (NIR spectroscopy) and acid insoluble ash (AIA) (Van Keulen and Young, 1977). One composited sample of faeces per animal was dried at 98°C for 48 h for DM determination while another was dried at 60°C for 48 h and milled for the analysis of Yb, Co, INDF, ash, N, NDFom and AIA.

Dry matter content of rumen solid fractions was determined by drying at 60°C for 72 h in an oven with forced air circulation and corrected for loss of volatiles according to Shiels et al. (1999). These samples were ground through a Wiley mill (1 mm screen) and analysed for ash, N, NDFom, ADFom and INDF.

Rumen fluid samples were analysed for ammonia N ((NH3-N) measured using the Olympus AU400 and the Thermo Electron Infinity Ammonia Liquid Stable Reagent kinetic method)) and lactic acid (using the Olympus AU400 and the L-Lactic Acid UV-method test kit (Boehringer Mannheim / R-Biopharm catalogue number 10139084035) with the enzyme D-Lactate Dehydrogenase (Boehringer Mannheim / R-Biopharm catalogue

number 10106941001). The concentration of VFA was measured using an automated gas chromatograph (Shimadzu Gas Chromatography GC-8A; Shimadzu Corporation, Kyoto 604-8511, Japan) (Ranfft, 1973).
Omasal digesta and faeces were analysed for Yb and Co according to Hart and Polan (1984). The INDF concentration of grass, faeces and digesta was determined by weighing 2 – 4 g material into 6μm pore size nylon bags and incubating in the rumen of two animals for 12 d according to Ahvenjärvi et al. (2000). Purine bases in the microbial pellet were determined according to the method of Zinn and Owens (1986) and modifications of Makkar and Becker (1999).

2.12 Statistical analysis
Grass intake, daily mean rumen fermentation parameters, degradability, nutrient flow and rumen evacuation data were analysed using PROC MIXED (SAS, 2003) appropriate for a 2 × 2 cross-over design experiment. The model had fixed effects for treatment, period and sequence group, and animal within sequence group as a random variable.

3. Results

3.1 Weather and grass production
Total rainfall for the months of April, May and June 2005 was 68, 89 and 25 mm, respectively, compared with the corresponding 20-year averages of 68, 56 and 72 mm. Total sunshine hours and mean daily ground temperatures were 132, 144 and 129, and 8.2, 10.3 and 15.2 ^{o}C, respectively compared with the corresponding 20–year averages of 134, 171 and 139 h, and 8.0, 10.6 and 13.1 ^{o}C. Grass DM yield for April, May and June was 1539, 1696 and 1677 kg DM/ha compared with the 5-year averages of 1692, 2313 and 1654 kg DM/ha.

3.2 Grass yield and chemical composition
The pre-cutting grass yield, sward height and chemical composition of the grass used is presented in Table 3.1. The mean pre-cutting grass DM yield and sward height was 831 kg DM/ha and 31 mm higher, respectively for the 38 than the 28 d RI treatment. The *in vitro* digestibility and chemical composition of the grass from both treatments was similar, except for the CP and WSC concentrations which were numerically lower and higher, respectively for the 38 than the 28 d RI treatment.

3.3 Intake and rumen fermentation
There was no difference (P>0.1) in dry matter intake (DMI) between the RI treatments (Table 3.2). Rumen pH tended to be higher (P=0.07) and total VFA concentration lower (P=0.08) for the 38 than the 28 d RI treatment. Concentrations of lactic acid or the molar proportions of VFA did not differ (P>0.1) due to RI. The concentration of ammonia in the rumen was higher (P<0.05) for the 28 than the 38 d RI treatment.

3.4 OM and NDFom flow and digestibility
There was no effect (P>0.1) of RI on omasal flow, ruminal digestibility or total tract digestibility of OM (Table 3.3). Neutral detergent fibre intake and total tract NDFom digestibility did not differ (P>0.1) between the RI treatments, whereas there was a tendency for NDFom flow to be higher (P=0.07) and ruminal NDFom digestibility to be lower (P=0.08) with the 38 than the 28 d RI treatment. Ruminal digestibility as a proportion of total tract digestibility was lower (P<0.05) for the 38 than the 28 d RI treatment.

3.5 Ruminal N metabolism
There was no difference (P>0.1) between the RI treatments in N, non-ammonia N (NAN) or microbial N (MN) flow (Table 3.4). Nitrogen intake (P<0.001) and non-ammonia non-microbial N (NANMN) (P<0.05) flow was higher and N flow expressed as a proportion of N intake tended to be lower (P=0.07) for the 28 than the 38 d RI treatment. NAN flow minus N intake tended to be lower (P=0.07) for the 38 than the 28 d RI treatment. Efficiency of microbial protein synthesis did not differ (P>0.1) between the RI treatments. There was a tendency for a higher apparent ruminal (P=0.07) and total tract (P=0.10) N digestibility with the 28 than the 38 d RI treatment.

3.6 In situ degradation
The 38 d RI treatment had a higher (P<0.05) 'D' fraction, a lower (P<0.01) undegradable fraction and a lower rate of degradation of DM (k_d) (P<0.001), and effective degradability (P<0.05) of DM than the 28 d RI treatment (Table 3.5). The rate of degradation of OM was higher (P<0.001) for the 28 than the 38 d RI treatment but there was no effect of RI on the 'W', 'D' and 'U' fractions. For N degradability, the 28 d RI treatment had a lower (P<0.05) 'W' fraction and a higher (P<0.05) 'D' fraction and rate of N degradation than the 38 d RI treatment. The N undegradable fraction or effective degradability did not differ (P>0.1) between the RI treatments. The NDFom 'D' and 'U' fractions tended to be higher (P=0.06) and the rate of NDFom degradation was higher

(P<0.01) for the 28 than the 38 d RI treatment. The PDIN (true protein absorbable in the small intestine when degradable N is limiting microbial protein synthesis in the rumen) concentration was higher (P<0.001) for the 28 than the 38 d RI treatment (75 *versus* 64 g/kg DM). The PDIE (true protein absorbable in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen) value was numerically higher for the 28 d RI treatment (101 versus 95 g/kg DM).

3.7 Rumen digesta kinetics

There was no effect (P>0.05) of RI treatment on the rumen pool size of DM, OM or N (Table 3.6). Rumen pool size of NDFom and DNDF (P<0.05) was higher with the 38 than the 28 d RI treatment. Fractional rate of intake (k_i) of NDFom and DNDF was higher (P<0.05), and of DM and OM tended to be higher (P=0.07) for the 28 compared to the 38 d RI treatment. There was no effect (P>0.1) of RI treatment on the fractional rate of passage (k_p) of NDFom, DNDF or INDF, whereas the differences seen with the fractional rate of degradation (k_d) reflected those seen with the k_i. The 28 d RI had a higher rate of degradation of DM, OM, (P<0.05), NDFom and DNDF (P<0.01) than the 38 d RI treatment.

4. Conclusion

Allowing a perennial ryegrass-based sward to grow for an additional 10 days, from 28 to 38 days in spring/early summer had relatively little or no adverse effect on intake, rumen fermentation and rumen or total tract digestion of OM and NDFom. Increasing RI reduced rumen ammonia concentrations and the amount of undegraded feed N leaving the rumen thus, potentially reducing N excretion to the environment.

Table 3.1: Pre-cutting dry matter yield (kg/ha) and sward height (mm), *in vitro* digestibility and chemical composition of the herbage used (g/kg DM unless otherwise stated) (S.D. in parentheses)

	Regrowth Interval	
	28d	38d
DM yield (kg/ha)	2727 (680.1)	3558 (879.8)
Sward height (mm)	146 (1.8)	177 (2.8)
Dry matter (g/kg)	200 (25.9)	210 (29.0)
in-vitro DMD (g/kg)	849 (17.1)	849 (18.5)
in-vitro DOMD (g/kg)	770 (20.2)	774 (15.6)
Ash	86 (6.8)	82 (5.9)
Crude protein	116 (17.3)	99 (16.3)
NDFom	406 (39.8)	410 (36.1)
ADFom	231 (17.5)	249 (18.3)
WSC	299 (25.1)	323 (24.3)
INDF	31 (4.0)	36 (8.1)

Table 3.2: Mean dry matter intake and rumen fermentation characteristics of beef steers offered zero-grazed perennial ryegrass of 28- and 38-d regrowth intervals

	Regrowth Interval			
	28d	38d	S.E.M.	P =
DM intake (kg/d)	8.64	8.42	0.169	0.211
DM intake (g/kg BW)	19.8	19.3	0.25	0.230
pH	6.65	6.78	0.037	0.065
Lactic acid (mg/l)	48.3	67.9	24.05	0.389
Ammonia (mg/l)	18.1	9.7	1.96	0.038
Total VFA (mmol/l)	104.1	96.7	2.24	0.079
Molar proportions (mmol/mol)				
Acetic acid	626	630	4.3	0.405
Propionic acid	201	199	2.8	0.647
Butyric acid	150	149	3.0	0.752
Valeric acid	24	27	0.6	0.342
Acetate:Propionate ratio	3.13	3.19	0.060	0.508

Table 3.3: Nutrient intake, omasal flows, ruminal and total tract digestibility in steers offered zero-grazed perennial ryegrass of 28- and 38-d regrowth intervals

	Regrowth Interval			
	28d	38d	S.E.M.	*P* =
OM				
Intake (kg/d)	7.89	7.73	0.159	0.292
OM flow (kg/d)	2.17	2.24	0.107	0.677
OMAD[1]	0.724	0.712	0.0155	0.528
OMTD[2]	0.878	0.879	0.0144	0.323
Total tract digestibility	0.856	0.864	0.0049	0.223
NDFom				
Intake (kg/d)	3.51	3.45	0.069	0.414
NDFom flow (kg/d)	0.668	0.839	0.067	0.072
Ruminal digestibility	0.809	0.757	0.0198	0.082
Total tract digestibility	0.819	0.826	0.0101	0.601
Ruminal/total digestibility	0.988	0.917	0.0181	0.024

[1] OM apparently digested in the rumen (OM intake – OM flow)
[2] OM truly digested in the rumen (OMAD + microbial OM flow), assuming the ash content of microbial DM was 0.1

Table 3.4: Nitrogen intake, omasal N, microbial N and NANMN flow (g/d) in steers offered zero-grazed perennial ryegrass of 28- and 38-d regrowth intervals

	Regrowth Interval			
	28d	38d	S.E.M.	*P* =
N intake (g/d)	160	133	2.7	<0.001
N flow (g/d)	150	139	7.3	0.235
NAN[1] flow (g/d)	140	131	6.7	0.346
N intake - NAN flow (g/d)	20	2	8.2	0.068
N flow/N intake	0.94	1.04	0.060	0.067
Microbial N flow (g/d)	99	107	6.1	0.423
NANMN[2] (g/d)	51	32	8.0	0.047
Microbial efficiency				
g of N/kg OMAD[3]	17.6	19.3	1.35	0.426
g of N/kg OMTD[4]	14.4	15.5	0.91	0.417
N digestibility				
Apparent ruminal	0.058	-0.043	0.0591	0.070
True ruminal[5]	0.680	0.751	0.0617	0.264
Total tract	0.753	0.727	0.0097	0.099

[1]NAN: non-ammonia N; [2]NANMN: non-ammonia non-microbial N; [3]OMAD: OM apparently digested in the rumen: [4]OMTD: OM truly digested in the rumen; [5]True ruminal N digestibility: 1 – [(N flow – microbial N)/N intake]

Table 3.5: *In situ* degradation of dry matter, organic matter, nitrogen and NDFom in steers offered zero-grazed perennial ryegrass of 28- and 38-d regrowth intervals

	Regrowth Interval			
	28d	38d	S.E.M.	*P* =
DM				
W[1]	0.507	0.507	0.0061	0.982
D[2]	0.461	0.478	0.0072	0.016
U[3]	0.033	0.015	0.0035	0.005
k_d[4]	0.063	0.043	0.0028	<0.001
ED[5]	0.79	0.76	0.008	0.045
OM				
W	0.490	0.471	0.0192	0.511
D	0.477	0.486	0.0081	0.340
U	0.033	0.043	0.0191	0.697
k_d	0.065	0.045	0.0029	<0.001
ED	0.83	0.79	0.023	0.445
N				
W	0.271	0.392	0.0474	0.018
D	0.671	0.541	0.0494	0.019
U	0.059	0.068	0.0060	0.307
k_d	0.081	0.057	0.0037	0.012
ED	0.72	0.71	0.027	0.321
NDFom				
D	1.049	1.004	0.0124	0.062
U	0.061	0.057	0.0027	0.064
k_d	0.064	0.048	0.0022	0.009

[1]W, instantly degradable fraction
[2]D, potentially degradable fraction
[3]U, undegradable fraction
[4]k_d, rate of degradation of fraction D (/h)
[5]ED, effective degradability

Table 3.6: Digestion kinetics of dry matter, organic matter, NDFom, DNDFom, INDFom and nitrogen in steers offered zero-grazed perennial ryegrass of 28- and 38-d regrowth intervals

	Regrowth Interval			
	28d	38d	S.E.M.	*P* =
DM				
Rumen pool size (kg)	4.39	4.76	0.211	0.193
k_i[1]	0.085	0.075	0.0032	0.066
k_p[2]	0.041	0.039	0.0021	0.501
k_d[3]	0.044	0.036	0.0016	0.022
OM				
Rumen pool size (kg)	3.57	3.97	0.181	0.162
k_i	0.096	0.083	0.0038	0.065
k_p	0.026	0.024	0.0023	0.435
k_d	0.070	0.059	0.0022	0.025
NDFom				
Rumen pool size (kg)	2.21	2.67	0.104	0.030
k_i	0.069	0.056	0.0026	0.023
k_p	0.013	0.014	0.0013	0.762
k_d	0.056	0.042	0.0022	0.009
DNDFom				
Rumen pool size (kg)	1.54	1.95	0.098	0.041
k_i	0.093	0.070	0.0041	0.020
k_p	0.011	0.012	0.0015	0.532
k_d	0.082	0.058	0.0034	0.009
INDFom				
k_p	0.017	0.017	0.0014	1.000
N				
Rumen pool size (kg)	0.166	0.159	0.0113	0.246

[1] k_i = Rate of intake
[2] k_p = Rate of passage
[3] k_d = Rate of digestion

EXPERIMENT 4: Intake, rumen fermentation, degradability and digestion kinetics in beef cattle offered autumn grass herbage differing in regrowth interval

1. Introduction

Production and efficient utilisation of high yields of high-quality grass herbage throughout its growing season are critical for cost-efficient beef production in many systems (O'Riordan *et al*., 2000). In Ireland the live weight gain of grazing cattle after late summer is generally lower than that achieved in the spring and mid-summer (Devaney *et al*., 1998). This decrease in live weight gain in the autumn may be due to the quantity and/or quality of the herbage on offer relative to nutrient requirements. It has been suggested that the digestible energy contained in autumn herbage is utilized less efficiently than in the spring or summer for live weight gain (Corbett *et al.,* 1966; Blaxter *et al*., 1971).

Environmental legislation and the rules of environmental schemes are progressively restricting the application of nitrogen (N) fertilizer, and grazing systems are becoming more extensive in Europe. Reducing N excretion to the environment by increasing N utilisation has become a major area of emphasis in ruminant research (Hoekstra *et al*., 2007). Lowering application rates of N fertilizer and increasing regrowth interval of grass herbage have been identified as grassland management tools to reduce crude protein (CP) concentration in herbage and increase the subsequent efficiency of N use by ruminants (Peyraud and Astigarraga, 1998; Hoekstra *et al*., 2007). O'Riordan (1997) has shown that extending the length of grazing rotations by an additional 7 d (21 vs. 28 d) resulted in increased herbage production, which was equivalent to the production response to an annual application of approximately 150 kg N fertilizer ha^{-1}. As regrowth interval increases, the nutritive value of herbage generally declines due to increased lignification, a decreased proportion of leaf: stem and a decrease in CP concentration (Delagarde *et al*., 2000). The *in vitro* digestibility of grass herbage in the autumn, however, has been found not to change between 3 and 9 to 10 weeks before decreasing (O'Riordan *et al*., 1998).

In comparison to published information on degradation rates in the rumen, there is a lack of knowledge on rates of passage of nutrients out of the rumen (Dijkstra *et al*., 2007). While a number of studies have examined the effects of grass maturity or regrowth interval on digestion in the rumen of grass silage (Rinne *et al*., 1997a and b; Cone *et al.,* 1999; Rinne *et al.,* 2002), there is a shortage of comparable data for fresh grass. A recent study examined the effects of regrowth interval of grass herbage in spring and early summer (Owens *et al*., 2008).

Extending grazing into late autumn and early winter can potentially reduce feed costs by replacing expensive silage and concentrate feeds by cheaper grazed grass. Thus, the aim of this experiment was to determine the effects of increasing the regrowth interval of grass herbage from 35 to 45 d in autumn on dry matter (DM) intake, ruminal concentrations of volatile fatty acids (VFA) and NH_3-N, *in situ* degradability of grass and the kinetics of digestion of grass herbage in beef cattle. The experiment was conducted under a zero-grazing rather than an *in situ* grazing regime to facilitate the management and accurate collection of data.

2. Material and methods

All procedures in the study were conducted under experimental licence from the Irish Department of Health and Children in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulation, 1994.

Experimental area

The study was carried out at Teagasc, Grange Beef Research Centre, Ireland (longitude 6^o 40' W; latitude 53^o 30' N; elevation 92 m asl) between 27 July and 7 October 2005. The soil type was a moderately well-drained Brown Earth of medium to high base content and of clay loam texture. Meteorological data were recorded at Grange Beef Research Centre. The 20-year (1982-2002) mean annual rainfall, duration of sunshine hours and daily temperature at 50 mm below ground level were, respectively, 867 mm, 1202 h and 9.0 ^oC. The 5-year (2001-2005) mean annual grass production at this location, according to O'Riordan (1997), was 10 576 kg DM ha^{-1}.

The experimental area (2 ha) consisted predominantly of a late-heading perennial ryegrass (*Lolium perenne*, cv. Melle*)* sward sown in 2002. It received 50 kg fertilizer-N in the form of urea on 7 February and applications of 25 and 50 kg ha^{-1} of P_2O_5 and K_2O, respectively, supplied as 250 kg ha^{-1} of a 0:10:20 compound fertilizer on 9 March. The area was used for a similar zero-grazing experiment in spring where 33 kg N ha^{-1} (urea) was applied (Owens *et al*., 2008). Before the start of the current experiment the area was mown and the cut herbage removed on 25 July.

Treatments
Treatments consisted of regrowth intervals of 35 and 45 d. The area was longitudinally divided into two plots, one for each regrowth interval and each plot was divided into thirteen individual 3 d subplots. It was designed so that each subplot would provide sufficient herbage for 3 d zero-grazing during the feeding study. For preparation of the experimental herbage, 46 d and 36 d prior to feeding, the herbage was harvested (commencing on 25 July and 4 August for the 45 and 35 d treatments, respectively), to a stubble height of 6 cm from the respective subplots using an Agria mower, removed and 33 kg N ha^{-1} as urea was applied. This sequence was repeated every 3 d to ensure the difference in regrowth interval was maintained for the two contrasting herbages.

Management of steers
Six ruminally fistulated Holstein-Friesian steers, fitted with 100-mm internal diameter ruminal cannulae, and with an average live weight (s.d. of mean) of 527 (18) kg at the start of the experiment, were used in a 2 (treatments) × 2 (periods) cross-over design. The second period followed immediately after the first period and each period consisted of 16 d with 6 d of dietary adaptation and 10 d of measurements. A short dietary adaptation period has been previously reported as being adequate for fresh grass diets (Stakelum and Dillon, 2003)
Steers were housed in a tie-stall barn with free access to water at all times. The grass regrowth was harvested daily at 11.00 h to a stubble height of 6 cm using an Agria mower and was stored without further chopping at 4 ^{o}C until required. The grass herbage was offered *ad libitum* in two equal daily meals at 08:30 and 20:30 h at proportionately 1.1 of the previous day's intake until d 5 and then subsequently offered at 0.95 of the average intake of d 3, 4 and 5 until the end of each period.

Sward measurement and grass sampling
Pre-cutting sward heights were measured daily using a rising plate meter (Jenquip, Fielding, New Zealand) and pre-cutting herbage mass was determined by weighing the grass and measuring the area harvested daily to the stubble height of 6 cm. Duplicate samples of herbage offered were obtained daily. Samples were stored at -20^{o}C until processing when samples were bulked for d 6-8, 9-12, 13-14 and 15-17 using a bowl chopper (Muller, Type MKT 204 Special, Saarbrucken, Germany) yielding a total of four samples per treatment per period for chemical analyses. The DM allowance of herbage offered daily was estimated by rapid DM determination of three 50-g representative samples per treatment using microwave drying for a period of 12 min. (O'Kiely, 1997).

Rumen evacuation
Rumen evacuations for determining rumen pool size and digesta kinetics were carried out prior to the morning feeding on d 7, and 3 h and 6 h after the morning feeding on d 8 and d 9, respectively. Rumen contents were collected into an 80-L non-insulated plastic container with a stainless steel grid 20 cm from the base to facilitate the separation of solid and liquid fractions with a tap at the base to assist the collection of liquid. Rumen liquid and solid fractions were weighed. A 500-mL sample of the liquid was obtained of which a 20-mL sub-sample was acidified with 0.5 mL 9M sulphuric acid. After sampling the liquid was returned to the rumen followed by the solid fraction, which was sampled regularly during this process. The resulting solid fraction sample was thoroughly mixed, a ~2.0 kg (equivalent to 0.06 of total solid fraction) sub-sample was obtained and the remainder was returned to the rumen. The evacuation and returning of contents to the rumen lasted 45 min per steer and the rumen was empty for 2-3 min. All samples were then stored at -20^{o}C.

Rumen fluid sampling
Rumen fluid samples of approximately 200 mL were collected through the rumen cannulae at 08:30, 10:30, 12:30, 14:30, 16:30, 18:30 and 20:30 h on d 10 of each period. This was achieved using a plastic tube (750-mm long closed at one end, 12 mm i.d. with holes of 2 mm diameter and 20 mm apart in the bottom 200 mm), which was attached to a vacuum system and collection vessel. The pH of rumen fluid was measured immediately after collection using an Orion digital pH meter (Orion, SA720; Thermo Fisher Scientific, Waltham, MA, USA) and glass electrode. A 20 ml sub-sample was acidified with 0.5 mL of 9M sulphuric acid and stored at –20 ^{o}C for subsequent analysis of VFA, lactic acid and NH_3-N concentrations.

Degradability in the rumen
Representative samples of herbage (about 1.5 kg fresh weight) from each treatment were dried at 40^{o}C for 48 h in a forced air oven and ground to pass a 2-mm screen. Degradability in the rumen was determined using the *in situ* nylon bag technique. On d 10 of each period, nylon bags (5 cm × 10 cm; 53 micron pore size, Ankom Technology Corporation, New York, USA) containing 2 g of dried herbage were incubated in duplicate in the rumen for 0, 2, 4, 8, 12, 24, 48 and 72 h. The undegradable neutral-detergent fibre (NDF) fraction was determined separately by incubating 20 bags per treatment in the rumen for 288 h (Robinson *et al*., 1986). The 0 h time point represented the bags which were not incubated in the rumen but were subsequently treated in the

same manner as the incubated bags. After removal from the rumen, bags were stored at -20 °C until thawing, stomaching in a bicarbonate solution and machine-washing. Dry matter content was determined in an oven with forced air circulation at 40 °C for 48 h. Dried bags were weighed to determine DM disappearance and residues were removed and analysed to determine CP, organic matter (OM) and NDF concentration in the DM.

Faecal sampling

Faecal grab samples (~500g) were obtained via rectal palpation three times daily at an interval of 4 h for d 11 to 14 (Ahvenjarvi *et al*., 2001) to determine total tract digestibility of the herbage using acid-insoluble ash (AIA) as a digestibility marker. Sampling started at 08:30 h and advanced 1 h each day to cover a 12-h period. These samples were stored at -20°C. At the end of the sampling period these samples were thawed and pooled on an equal weight basis, per steer per period.

Calculations

Passage and digestion kinetics of dietary constituents from the data on rumens evacuation were calculated according to Robinson *et al.* (1987) using the following equations:

Rate of intake (k_i) = intake (kg 24 h^{-1}) / rumen pool (kg)

Rate of passage (k_p) = faecal output (kg 24 h^{-1}) / rumen pool (kg)

Rate of digestion (k_d) = k_i - k_p

The instantly degradable soluble fraction was estimated as the fraction disappearing during washing. The degradability data of the grass herbage were calculated according to the first order model of Robinson *et al.* (1986). This model includes an insoluble potentially degradable fraction, which is degraded at a constant rate of degradation (k_d), and with an undegradable fraction. The effective degradability was determined according to Salaün *et al.* (1999) using an assumed outflow rate of 0.06 h^{-1}. The values for protein truly digestible in the small intestine were calculated using data obtained from degradability and chemical analysis according to Vérité and Peyraud (1989).

Chemical analyses

The DM content of the grass was determined after drying at 98 °C for 16 h in a forced air oven. These pooled samples were freeze-dried and ground through a Wiley mill (1-mm screen) for analysis of *in vitro* DM digestibility (Tilley and Terry, 1963) with the modification that the fluid residue was isolated by filtration rather than centrifugation, NDF and acid-detergent fibre (ADF) concentrations (Van Soest *et al*., 1991), ash by burning in a muffle furnace at 550 °C for 5 h, N using a Dumas-type N analyser (Leco FP-428; Leco Corporation, St Joseph, MI; CP = N × 6.25), water-soluble carbohydrates (WSC) (Anthrone method; Thomas, 1977) and AIA (Van Keulen and Young, 1977). One composite sample of faeces per steer was dried at 98 °C for 48 h for determination of DM content while another was dried at 60 °C for 48 h and milled for the analysis of indigestible NDF (INDF), ash, N, NDF and AIA. The INDF concentration of grass, faeces and digesta was determined according to Ahvenjarvi *et al*. (2000).

The DM content of the rumen solid fraction was determined by drying at 60°C for 72 h in a forced air oven and corrected for loss of volatiles according to Shiels *et al*. (1999). These samples were ground through a Wiley mill (1-mm screen) and analysed for ash, N, NDF, ADF and INDF.

Rumen fluid samples were analysed for NH3-N concentration using the Olympus AU400 and the Thermo Electron Infinity Ammonia Liquid Stable Reagent kinetic method and lactic acid concentration using the Olympus AU400 and the L-Lactic Acid UV-method test kit (Boehringer Mannheim / R-Biopharm catalogue number 10139084035) with the enzyme D-Lactate Dehydrogenase (Boehringer Mannheim / R-Biopharm catalogue number 10106941001). The concentration of VFAs was measured using an automated gas chromatograph (Shimadzu Gas Chromatography GC-8A, Shimadzu Corporation, Manchester, UK) (Ranfft, 1973).

Statistical analyses

Herbage intake, daily mean rumen fermentation variables, degradability and rumen evacuation data were analysed using the general linear model procedure (PROC GLM) (SAS, 2003) appropriate for a 2 × 2 cross-over design experiment. The model had terms for treatment, period and steer.

3. Results

There were no treatment × period interactions for any variables.

Weather and herbage production

Total rainfall for the months of July, August, September and October 2005 were 91, 34, 57 and 104 mm, respectively, compared with the corresponding 20-year averages of 52, 79, 66 and 90 mm. Total sunshine hours and mean daily soil temperatures at 50 mm below ground level were 90, 141, 102 and 45, and 17.3, 15.7, 13.7

and 10.8 ^{o}C, respectively, compared with the corresponding 20–year averages of 135, 138, 112 and 88 h and 15.8, 14.7, 12.0 and 8.6^{o}C. Dry matter yields of herbage in July, August, September and October were 1364, 1209, 869 and 390 kg DM ha^{-1}, respectively, compared with the corresponding 5-year averages of 1532, 1228, 745 and 336 kg DM ha^{-1}.

Herbage yield and composition
The DM yield and chemical composition of herbage of the perennial ryegrass-based sward is presented in Table 4.1. The mean pre-harvesting DM yield of herbage and sward height were 615 kg DM ha^{-1} and 19 mm higher, respectively, for the 45 d than the 35 d regrowth interval treatments. Both treatments had high *in vitro* OMD values and similar ash, NDF and ADF concentrations. The CP and WSC concentrations were 27 and 22 g kg^{-1} higher and lower, respectively, for the 35 d compared to the 45 d regrowth interval treatments.

Intake, rumen fermentation and total tract digestibility
There was no effect of regrowth interval treatment on DM intake, rumen pH, concentrations of lactic acid or total VFA, or on the molar proportions of acetate, propionate and butyrate in the rumen (Table 4.2). The concentration of NH_3-N (P<0.01) and the molar proportion of valeric acid (P<0.05) in the rumen were higher for the 35 d than the 45 d treatment. The apparent total tract digestibility of DM, OM (P<0.01), N (P<0.001) and NDF (P<0.05) was higher for the 35 d compared to the 45 d treatment (Table 4.3).

In situ degradation
There was no effect of regrowth interval treatment on the instantly degradable soluble fraction, insoluble potentially degradable fraction and undegradable fraction, or on the rate of degradation (k_d) of DM, OM or N (Table 4.4). There was a tendency (P=0.053) for the effective degradability of DM to be lower at the longer regrowth interval. The effective degradability of N was high and averaged 0.73. Neutral-detergent fibre concentration of the insoluble potentially degradable fraction and undegradable fraction and k_d of NDF did not differ between the treatments. The values for protein truly digestible in the small intestine of the grass herbage samples incubated in the rumen are presented in Table 4.5. The amount of absorbable microbial protein that could be synthesised in the rumen, when rumen degradable-N is limiting (P<0.01) and true protein absorbable in the small intestine when degradable N is limiting microbial protein synthesis in the rumen (P<0.001) concentrations were higher for the 35 d than the 45 d treatment. There was no effect of regrowth interval treatment on the amount of absorbable microbial protein that could be synthesised in the rumen, when rumen fermentable energy (expressed as OM) is limiting or true protein absorbable in the small intestine when rumen fermentable energy (expressed as OM) is limiting microbial protein synthesis in the rumen.

Rumen digesta kinetics
There was no effect of regrowth interval treatment on the rumen pool size of DM, OM, NDF, ADF or N (Table 4.6). The fractional rate of intake (k_i) of DM, OM or digestible NDF was unaffected by treatment, whereas the k_i of NDF was higher (P<0.05) for the 35 d compared to the 45 d treatment. The k_d of DM and NDF was higher (P<0.05) for the 35 d than the 45 d treatment, whilst the rate of passage (k_p) of DM (P<0.01) and OM (P<0.05) was higher for the 45 d than the 35 d treatment.

4. Conclusions

The results from this study suggest that allowing perennial ryegrass-based swards to grow for an additional 10 d from 35 to 45 d in autumn had no adverse effect on intake or rumen fermentation. Increasing regrowth interval reduced rumen NH_3-N concentrations and thus potentially reducing N excretion to the environment. There was no effect of increasing regrowth interval on rumen *in situ* degradability of DM, OM, N and NDF, whereas there was a reduction in the rate of digestion of NDF using the rumen evacuation technique.

Table 4.1 Pre-cutting dry matter yield and sward height, *in vitro* digestibility and concentrations of ash, neutral-detergent fibre (NDF), acid-detergent fibre (ADF), water-soluble carbohydrates (WSC) and indigestible neutral-detergent fibre (INDF) in the herbage offered. Standard deviation of the mean is in parentheses.

	Regrowth Interval	
	35 days	**45 days**
DM yield (kg ha^{-1})	2278 (556.9)	2893 (546.2)
Sward height (mm)	100 (16.0)	119 (9.2)
Dry matter content (g kg^{-1})	172 (2.3)	174 (2.1)
in-vitro OMD[1]	0.808 (0.0152)	0.813 (0.0094)
in-vitro DOMD[2]	0.719 (0.0152)	0.724 (0.0107)
Ash concentration (g kg^{-1} DM)	110 (6.3)	109 (5.2)
Crude protein concentration (g kg^{-1} DM)	192 (18.9)	165 (16.3)
NDF concentration (g kg^{-1} DM)	451 (23.7)	440 (8.3)
ADF concentration (g kg^{-1} DM)	278 (19.6)	280 (13.3)
WSC concentration (g kg^{-1} DM)	147 (16.2)	169 (19.6)
INDF concentration (g kg^{-1} DM)	31(4.2)	36 (3.6)

[1]OMD, organic matter digestibility
[2]DOMD, digestible organic matter in the dry matter

Table 4.2 Mean dry matter (DM) intake and rumen fermentation characteristics of beef steers offered zero-grazed perennial ryegrass herbage after 35- and 45-day regrowth intervals

	Regrowth interval		**s.e. of mean**	**Level of significance**[†]
	35 days	**45 days**		
Intake				
DM intake (kg d^{-1})	9.42	9.04	0.419	NS
DM intake (g kg^{-1} live weight)	17.9	17.2	0.82	NS
Rumen fermentation				
pH	6.75	6.75	0.008	NS
Lactic acid concentration (mg L^{-1})	63.7	71.6	3.63	NS
Ammonia-N concentration (mg L^{-1})	158.3	116.7	7.09	**
Total volatile fatty acid concentration (mmol L^{-1})	96.5	91.4	2.01	NS
Molar proportions				
Acetic acid	0.654	0.663	0.0023	NS
Propionic acid	0.171	0.176	0.0015	NS
Butyric acid	0.146	0.138	0.0020	NS
Valeric acid	0.029	0.024	0.0008	*
Acetate: propionate ratio	3.82	3.76	0.047	NS

[†]NS, not significant; *, $P<0.05$; **$P<0.01$

Table 4.3 Apparent digestibility of dry matter, organic matter, nitrogen and neutral-detergent fibre (NDF) of zero-grazed perennial ryegrass herbage of 35- and 45-days regrowth intervals offered to steers

	Regrowth interval		**s.e. of mean**	**Level of significance**[†]
	35 days	**45 days**		
Apparent digestibility of				
Dry matter	0.833	0.800	0.0036	**
Organic matter	0.856	0.828	0.0034	**
Nitrogen	0.838	0.779	0.0022	***
NDF	0.861	0.833	0.0066	*

[†]*, $P<0.05$; **, $P<0.01$, ***, $P<0.001$

Table 4.4 *In situ* of instantly degradable fraction (W), potentially degradable fraction (D), undegradable fraction (U), rate of degradation of fraction D (k_d, per h) and effective degradability (ED) of dry matter, organic matter, nitrogen and neutral-detergent fibre (NDF) of zero-grazed perennial ryegrass herbage of 3- and 45-days regrowth intervals

	Regrowth interval		s.e. of mean	Level of significance[†]
	35 days	45 days		
Dry matter				
W	0.427	0.409	0.0088	NS
D	0.523	0.537	0.0116	NS
U	0.050	0.054	0.0042	NS
k_d	0.071	0.065	0.0050	NS
ED	0.71	0.68	0.008	P=0.05
Organic matter				
W	0.392	0.373	0.0087	P=0.09
D	0.561	0.577	0.0114	NS
U	0.046	0.051	0.0073	NS
k_d	0.071	0.066	0.0066	NS
ED	0.69	0.67	0.009	NS
Nitrogen				
W	0.438	0.349	0.0444	NS
D	0.520	0.608	0.0437	NS
U	0.043	0.042	0.0048	NS
k_d	0.102	0.084	0.0066	P=0.05
ED	0.76	0.70	0.023	P=0.06
NDF				
D	0.991	1.057	0.0348	NS
U	0.072	0.081	0.0079	NS
k_d	0.067	0.066	0.0049	NS

[1†]NS, non-significant

Table 4.5 The dietary protein undegraded in the rumen, but truly digestible in the small intestine (PDIA), the amount of absorbable microbial protein that could be synthesised in the rumen, when rumen degradable N is limiting (PDIMN), the amount of absorbable microbial protein that could be synthesised in the rumen, when rumen fermentable energy (OM) is limiting (PDIME), true protein absorbable in the small intestine when degradable N is limiting microbial protein synthesis in the rumen (PDIN) and true protein absorbable in the small intestine when rumen fermentable energy (OM) is limiting microbial protein synthesis in the rumen (PDIE) (all g kg^{-1} DM) of zero-grazed perennial ryegrass herbage of 35- and 45-days regrowth intervals.

	Regrowth interval		s.e. of mean	Level of significance[†]
	35 days	45 days		
PDIA	37.7	40.8	3.2	NS
PDIMN	81.6	63.7	2.46	**
PDIME	62.9	63.4	0.44	NS
PDIN	119.3	104.5	0.74	***
PDIE	100.6	104.1	3.22	NS

[†] NS, non significant; **, $P<0.01$, ***, $P<0.001$

Table 4.6 Digestion kinetics (k_i, rate of intake; k_p, rate of passage; and k_d, rate of digestion) of dry matter (DM), organic matter (OM), neutral-detergent fibre (NDF), digestible neutral-detergent fibre (DNDF), indigestible neutral-detergent fibre (INDF, k_p only), acid-detergent fibre (ADF, rumen pool size only) and nitrogen (N, rumen pool size only) in steers offered zero-grazed perennial ryegrass herbage of 35- and 45-day regrowth intervals

	Regrowth interval		**s.e. of mean**	**Level of significance**
	35 days	**45 days**		
DM				
Rumen pool size (kg)	6.54	6.30	0.203	NS
k_i[2]	0.061	0.061	0.0011	NS
k_p[3]	0.012	0.015	0.0005	**
k_d[4]	0.049	0.047	0.0005	*
OM				
Rumen pool size (kg)	5.30	5.26	0.253	NS
k_i	0.067	0.065	0.0019	NS
k_p	0.011	0.013	0.0004	*
k_d	0.056	0.052	0.0018	NS
NDF				
Rumen pool size (kg)	3.03	3.12	0.146	NS
k_i	0.060	0.054	0.0020	*
k_p	0.010	0.011	0.0005	NS
k_d	0.050	0.044	0.0018	*
DNDF				
Rumen pool size (kg)	1.91	1.99	0.181	NS
k_i	0.089	0.078	0.0069	NS
k_p	0.009	0.010	0.0011	NS
k_d	0.080	0.069	0.0064	NS
INDF				
k_p	0.011	0.013	0.0009	NS
ADF				
Rumen pool size (kg)	2.13	2.18	0.175	NS
N				
Rumen pool size (kg)	0.245	0.229	0.0104	NS

[1] NS, non-significant; *, $P< 0.05$; **, $P<0.01$

EXPERIMENT 5: Intake, growth, carcass traits, digestion, rumen fermentation and blood metabolites in steers offered different concentrate energy sources as supplements to grass silage or *ad-libitum*

1. Introduction

In addition to cereals, a wide variety of other feed ingredients, are available and used extensively in beef rations in Ireland. In practice, this means that isoenergetic and isonitrogenous concentrates can contrast from being high in starch (of varying rumen degradability) to being high in digestible fibre and consequently, providing a variable balance of nutrients for absorption. Due to changing relative costs of feeds and changing beef production systems, higher levels of concentrate feeding to beef cattle may be desirable in particular circumstances. Of particular concern when feeding high-grain diets however, is the excessively rapid fermentation of the high levels of starch to organic acids resulting in a risk of acidosis. There are inconsistent effects of replacing starch with digestible fibre in the concentrate on grass silage intake and the resultant performance of beef cattle (McGee, 2005). There is a paucity of data comparing concentrate energy sources offered at both moderate and high feeding levels with grass silage, particularly for late-maturing continental crossbred, finishing steers.

The objective of this study was to examine the effects of concentrate ingredient composition and feeding level on intake, growth, carcass traits, diet digestibility, rumen fermentation and plasma metabolite concentrations in finishing steers.

2. Materials and Methods

Animals and treatments

One hundred and twenty Continental crossbred steers initially offered grass silage *ad libitum* and 1.0 kg freshweight of a barley-based concentrates were weighed on two consecutive days. The average of these two weights was taken as the start weight (509 (s.d. 65.0) kg live weight). They were then blocked by breed and live weight and within blocks assigned at random to one of 8 treatments within a four (concentrate energy source) by two (feeding level) factorial arrangement of treatments (n = 15 / treatment). The four concentrates were 1. rapidly fermentable starch-based (barley-based) (RFS), 2. slowly fermentable starch-based (maize-based) (SFS), 3. rapidly fermentable starch plus fibre-based (RFS+F) and 4. fibre-based (pulps-based) (F) (Table 5.1). They were formulated to contain similar concentrations of energy and crude protein (CP). The two feeding levels were 1. grass silage *ad libitum* supplemented with 5 kg concentrate (fresh weight) per head daily and 2. *ad libitum* concentrates plus 5 kg fresh weight of grass silage per head daily. All animals had free access to water. Prior to initiation of the experiment, all animals were vaccinated against Parainfluenza 3 virus, Bovine Respiratory Syncytial virus, Mannheimia haemolytica (bacteria), infectious bovine rhinotracheitis and treated for ecot- and endo-parasites.

Feeds, feeding and blood sampling

Concentrate supplementation was introduced gradually with the adaptation phase for the supplemented and *ad libitum* concentrate treatments lasting approximately 12 and 40 days, respectively. The silage was from a predominantly perennial ryegrass sward harvested on 31 May. Grass was precision-chop harvested with minimal wilting, ensiled in horizontal silos and rolled thoroughly to ensure expulsion of air, before sheeting with 2 layers of polythene, and covered with tyres. For animals on the grass silage plus concentrate supplement treatments, fresh silage was offered in sufficient quantities to allow a refusal of 50-100 g/kg intake. Daily feed intake was obtained by weighing the quantities of feed offered *ad libitum* (silage for the supplemented treatments and concentrates for the *ad libitum* treatments) and subsequent refusals over 4 consecutive days each week. Silage refusals were discarded twice weekly. The concentrate supplements were offered once daily before feeding the silage in the morning. Representative samples of grass silage offered, silage refusals and concentrates offered were obtained three times, once and twice weekly, respectively. All feed samples were stored frozen at -20^{o}C until subsequent sub-sampling and chemical analysis.

The experimental animals were housed in a slatted floor shed in pen groups, with pens within treatments located in different parts of the shed. Live weight was recorded regularly throughout and on two consecutive days at the end of the study. On three separate occasions during the study two blood samples were obtained by jugular venipuncture using separate vacutainers containing lithium heparin and sodium fluoride-EDTA K3 as anticoagulants from each animal immediately before and 6 hours after the morning feeding for plasma metabolite analysis. For logistical reasons, blood sampling on each occasion was carried out over two days (day 30 and 31, 65 and 66, 107 and 114) with samples obtained from 0.5 of the animals on each day.

Slaughter and carcass assessment
Animals were slaughtered over two consecutive days in a commercial meat plant. After slaughter, cold carcass weight (0.98 × hot carcass weight) and weight of perinephric plus retroperitoneal fat were recorded. Carcasses were graded for conformation and fatness according to the European Union Beef Carcass Classification Scheme (Commission of the European Communities, 1982). Killing-out rates were calculated as the proportion of cold carcass weight to pre-slaughter live weight. Carcass gains were calculated as the difference between the final carcass weight and proportionately 0.53 of the initial live weight (Drennan et al., 2005). The duration of the study was 146 days.

Diet apparent digestibility
Apparent digestibility of the grass silage plus supplement diets was determined using 12 Holstein-Friesian steers (297 (s.d. 25.6) kg mean initial live weight) in two consecutive runs (n = 6 per treatment). The animals were fed the test diets at a restricted level of 0.85 *ad libitum* intake but at the same ratio of silage to concentrate as that consumed by the animals in the growth study. In a subsequent experiment also using 12 Holstein-Friesian steers (417 (s.d. 29.4) kg mean initial live weight) in two consecutive runs the apparent digestibility of the *ad libitum* concentrate diets were determined as described by Moloney and O'Kiely (1995). The silage, concentrates and faeces were collected and processed over a 10-d period according to the methods of Moloney and O'Kiely (1995).

Rumen fermentation and blood metabolites
Rumen fermentation parameters of the grass silage plus concentrate supplemented diets were determined using four rumen-fistulated Holstein-Friesian steers (661 (s.d. 13.0) kg mean initial live weight) in a 4 (diets) × 4 (periods) Latin square design experiment. The animals were fed the test diets at the same ratio of silage to concentrate as that consumed by the animals in the growth study. In a subsequent Latin square design experiment the same rumen fistulated steers, were used to evaluate the four concentrate energy sources offered *ad libitum* as per the production study. Both experiments consisted of 14-day periods comprising 10 days diet adaptation and 4 days sampling. On day 11, rumen fluid samples were obtained as described by Owens et al. (2008) at 0, 1, 2, 4, 6, 8, 12, 16 and 24 h post-feeding for the supplemented concentrate comparison. Corresponding sampling times for the *ad libitum* concentrate feeding comparison were 0, 2, 4, 6, 8, 12 and 24 h. On day 14, two blood samples were obtained by jugular venipuncture from each animal immediately before the morning feeding and subsequently 3 and 6 h later for plasma metabolite analysis.

Chemical analysis
Dry matter (DM) of the concentrate and silage used in the feeding and digestibility studies was determined by drying to a constant weight at 98^{o}C and 40^{o}C, respectively. Representative samples of the faeces collected in the digestibility study were dried at 40^{o}C. Following drying all samples were subsequently ground through a 1 mm screen and analysed for dry matter digestibility (DMD), acid detergent fibre (ADF) and neutral detergent fibre (NDF), crude protein, neutral cellulase digestibility, starch, oil A. Expressed silage juice was used to determine pH, concentrations of lactic acid, volatile fatty acids (acetic, propionic and butyric), ethanol, residual water soluble carbohydrate and ammonia-N as described by Owens et al. (2008). Concentrations of plasma beta-hydroxybutyrate (βHB), urea and glucose were analysed as described by Owens et al. (2008).

Statistical analysis
Data were statistically analysed using the general linear models procedure of the Statistical Analysis System Institute (SAS, 2003). The production study data were analysed as a 4 × 2 factorial using a model which had terms for concentrate energy source, feeding level and concentrate energy source × feeding level interaction. Pen was used as the experimental unit for analysis of data pertaining to feed intake and FCE. Analysis of variance was also carried out on the digestibility data and analysis of variance appropriate to a Latin Square design with repeated measures was carried out on rumen fermentation data. When significant effects due to treatments were detected, mean separation of treatment means was conducted by the PDIFF option. Least-square means are reported with standard errors. Three animals were removed from the production growth study for reasons not related to the treatments and are omitted from all analyses.

3. Results

Feed analysis
The ingredient composition of the concentrates and chemical analysis of the grass silage and concentrates used are presented in Table 5.1. The silage used had moderate DM and CP and a relatively high in vitro DMD. It was well preserved as shown by its high proportion of lactic acid within fermentation acids and its low pH and moderate ammonia-N value. The DM was lower for the RFS than the other concentrates. Crude protein

concentration was lower for the F and RFS+F than RFS and SFS. NDF and ADF concentrations were lowest for RFS and SFS, RFS+F and F in that order. Measures of *in vitro* digestibility were relatively similar for the four concentrates.

Intake, performance, slaughter traits and blood metabolites

There were no significant concentrate × feeding level interactions for intake, performance, slaughter traits or blood metabolites (Table 5.2). Concentrate energy source had no effect ($P>0.05$) on intake, slaughter weight, live weight gain, kill-out proportion, carcass weight, estimated carcass gain, FCE, carcass conformation score, carcass fat score or perinephric plus retroperitoneal fat weight or proportion. There was no difference ($P>0.05$) in total DM intake between the supplemented and *ad libitum* fed treatments. Increasing feeding level increased ($P<0.001$) FCE, final live weight, carcass weight and carcass fat measurements but had no effect ($P>0.05$) on kill-out proportion or carcass conformation score. Plasma βHB concentrations were lower ($P<0.001$) for SFS than the other concentrates, which were similar ($P>0.05$). Concentrations of βHB ($P<0.001$) and urea ($P<0.01$) were higher for the *ad libitum* than the supplemented feeding level.

Diet apparent digestibility

When offered as a supplement the in vivo dietary DM and CP digestibility did not differ ($P>0.05$) between the concentrates (Table 5.3). Digestibility of organic matter was lower ($P<0.05$) for SFS than F with RFS and RFS+F being intermediate ($P>0.05$). Digestibility of NDF was higher ($P<0.001$) for F than the other three concentrates, which did not differ ($P>0.05$), whereas that of ADF was lower ($P<0.05$) for RFS than F and RFS+F, with SFS being intermediate ($P>0.05$). When offered *ad libitum*, the digestibility of DM and OM did not differ ($P>0.05$) between the concentrates. Digestibility of NDF was lower ($P<0.01$) for RFS than F with SFS and RFS+F intermediate, and not different ($P>0.05$) from each other. Digestibility of ADF was lower ($P<0.05$) for RFS and SFS than RFS+F and F, whereas that of CP was lower ($P<0.05$) for F than RFS with SFS and RFS+F intermediate, and not different ($P>0.05$) from each other.

Rumen fermentation and blood metabolites

Rumen fermentation and blood metabolite results are presented in Table 5.4. When offered as supplements to grass silage, there was no effect ($P>0.05$) of concentrate type on rumen pH, ammonia, lactic acid or total VFA concentrations or molar proportions of acetate, propionate, butyrate or valerate. When offered *ad libitum*, rumen pH or total VFA concentrations and molar proportion of butyrate did not differ ($P>0.05$) between the concentrates but the molar proportion of acetate and valerate was lower ($P<0.05$) for RFS and SFS than RFS+F and F, and the molar proportion of propionate was higher ($P<0.05$) for RFS than RFS+F and F, with SFS being intermediate ($P>0.05$). Rumen ammonia concentrations were highest ($P<0.05$) for SFS and lowest for RFS+F and F with RFS being intermediate ($P>0.05$). Plasma βHB, urea and glucose did not differ ($P>0.05$) between the concentrates at either feeding level.

4. Conclusion

In conclusion, intake, production and carcass traits were unaffected by concentrate energy source, whereas increasing concentrate feeding level increased all production and carcass traits except kill-out proportion and carcass conformation. Concentrate energy source had no effect on rumen pH or fermentation parameters when offered as a supplement to grass silage but significantly altered the end products of rumen fermentation when offered *ad libitum*. Implications are that beef producers have widespread opportunity to source cost-effective concentrate supplements.

Table 5.1. Ingredient and chemical composition of the grass silage and concentrates at feeding

	Grass silage	Concentrate energy source			
		RFS	SFS	RFS+F	F
Ingredient composition (g/kg)					
Barley		843		409	
Maize meal			598		
Maize gluten feed			290		
Soyabean meal		73	30	108	135
Citrus pulp				215	377
Beet pulp (molassed)				195	420
Molasses (cane)		50	50	50	45
Minerals / vitamins		35	32	24	24
Chemical composition					
Dry matter (g/kg)	206 (22.4)	819 (6.3)	864 (7.7)	850 (8.1)	878 (14.6)
Crude protein (g/kg DM)	145 (6.9)	148 (3.1)	147 (9.6)	143 (8.6)	131 (5.6)
Ash (g/kg DM)	96 (7.0)	66 (6.1)	72 (2.7)	76 (9.6)	90 (10.9)
Neutral detergent fibre (g/kg DM)	556 (28.3)	158 (8.6)	211 (13.8)	242 (33.2)	281 (27.0)
Acid detergent fibre (g/kg DM)	357 26.5)	54 (6.3)	74 (13.7)	138 (21.3)	182 (16.1)
Starch (g/kg DM)	-	475 (18.9)	375 (26.3)	178 (45.8)	19 (10.9)
Sugar (g/kg DM)	-	69 (9.7)	76 (19.8)	147 (23.5)	194 (50.5)
Oil B (g/kg DM)	-	28 (1.4)	39 (2.8)	21 (2.6)	15 (2.1)
Neutral cellulase digestibility (g/kg DM)	-	875 (16.7)	861 (44.2)	868 (10.9)	853 (29.0)
In vitro DM digestibility (g/kg)	719 (15.6)	916 (4.7)	930 (8.2)	929 (14.2)	937 (13.4)
In vitro OM digestibility (g/kg)	704 (14.5)	914 (5.1)	928 (8.8)	929 (13.7)	938 (11.4)
Ammonia (mg/100ml)	96 (7.0)	-	-	-	-
Lactic acid (g/L)	14 (5.9)	-	-	-	-
Ethanol (g/L)	5 (2.2)	-	-	-	-
Acetic acid (g/L)	7 (1.4)	-	-	-	-
Propionic acid (g/L)	0.4 (0.29)	-	-	-	-
Butyric acid (g/L)	0.4 (0.48)	-	-	-	-
Water soluble carbohydrates (g/kg DM)	10 (2.0)	-	-	-	-

[1]RFS = Rapidly fermentable starch; [2]SFS = Slowly fermentable starch; [3]RFS+F = Rapidly fermentable starch + Fibre; [4]F, Fibre.

Table 5.2. Effects of concentrate energy source and feeding level on feed intake, growth and carcass measurements in finishing steers

	Concentrate energy source (C)					Feeding level (F)			Significance [5]	
	RFS[1]	SFS[2]	RFS + F[3]	F[4]	s.e.m.	Supplemented	*Ad libitum*	s.e.m.	C	F
Daily dry matter (DM) intake										
Silage (kg)	3.8	4.0	3.8	3.6	0.19	5.8	1.8	0.14	NS	***
Concentrate (kg)	6.5	6.5	6.9	6.5	0.15	4.3	8.9	0.11	NS	***
Total (kg)	10.2	10.5	10.7	10.1	0.26	10.1	10.6	0.18	NS	NS
Total (g/kg live weight)	17.6	18.3	18.4	17.7	0.38	17.8	18.2	0.27	NS	NS
Final live weight (kg)	659	650	658	640	6.0	636	668	4.2	NS	***
Live weight gain (g/day)	1043	985	1032	911	40.3	884	1102	28.1	NS	***
Carcass weight (kg)	354	352	354	345	4.0	342	361	2.8	NS	***
Carcass gain (g/day)	582	570	584	520	27.2	497	631	18.9	NS	***
Kill-out proportion (g/kg)	537	541	540	539	4.2	537	541	2.93	NS	NS
Carcass conformation [6]	3.06	2.86	2.97	3.00	0.112	2.98	2.97	0.079	NS	NS
Carcass fat score [7]	3.47	3.35	3.53	3.46	0.105	3.29	3.68	0.074	NS	***
Perinephric + retroperitoneal fat (kg)	10.7	11.5	10.1	10.7	0.55	9.2	12.4	0.38	NS	***
Perinephric + retroperitoneal fat (g / kg carcass)	30.0	33.0	28.0	30.8	1.61	26.7	34.2	1.12	NS	***
Feed conversion efficiency										
g carcass / kg total DM intake	56.8	54.6	55.6	52.0	2.25	49.9	59.6	1.59	NS	***
Plasma metabolites (mmol / l)										
Beta-hydroxybutyrate	0.36[a]	0.30[b]	0.35[a]	0.35[a]	0.010	0.31	0.37	0.007	***	***
Urea	3.78	3.57	3.58	3.73	0.099	3.53	3.80	0.069	NS	**
Glucose	4.38	4.26	4.34	4.18	0.063	4.29	4.29	0.043	NS	NS

[1]RFS = Rapidly fermentable starch; [2]SFS = Slowly fermentable starch; [3]RFS+F = Rapidly fermentable starch + Fibre; [4]F, Fibre.

[5]No C × F interactions; [6, 7] EU Beef Carcass Classification Scheme: [6] Scale 1 (poorest) to 5 (best); [7] 1 (leanest) to 5 (fattest).

Table 5.3. Effects of concentrate energy source and feeding level on diet apparent digestibility and rumen degradability in steers

	Feeding level											
	Supplemented				s.e.m.	Sig.	*Ad libitum*				s.e.m.	Sig.
Concentrate energy source	RFS[1]	SFS[2]	RFS + F[3]	F[4]			RFS	SFS	RFS + F	F		
Digestibility (g/kg)												
Dry matter	739	727	748	757	8.6	P=0.07	810	777	799	779	16.9	NS
Organic matter	766^{ab}	752^{a}	774^{ab}	786^{b}	7.6	*	843	808	838	833	14.6	NS
Neutral detergent fibre	669^{a}	660^{a}	693^{a}	738^{b}	10.5	***	626^{a}	681^{ab}	745^{bc}	791^{c}	30.0	**
Acid detergent fibre	476^{a}	526^{ab}	617^{b}	601^{b}	35.2	*	561^{a}	557^{a}	771^{b}	791^{b}	21.7	*
Crude protein	643	619	604	616	16.7	NS	744^{a}	642^{bc}	689^{ab}	597^{c}	29.9	*

[1]RFS = Rapidly fermentable starch; [2]SFS = Slowly fermentable starch; [3]RFS+F = Rapidly fermentable starch + Fibre; [4]F, Fibre.

Table 5.4. Effects of concentrate energy source and feeding level on mean rumen fermentation variables and plasma metabolite concentrations in steers

	Feeding level											
	Supplemented				s.e.m.	Sig.	*Ad libitum*				s.e.m.	Sig.
Concentrate energy source	RFS[1]	SFS[2]	RFS + F[3]	F[4]			RFS	SFS	RFS + F	F		
Rumen Fluid												
pH	6.59	6.77	6.58	6.62	0.041	NS	6.27	6.38	6.29	6.31	0.070	0.063
Ammonia (mg/l)	61	57	55	50	5.1	NS	74^{ab}	87^{b}	51^{a}	53^{a}	7.5	*
Total volatile fatty acids (mmol/l)	141	137	144	142	3.4	NS	116	107	123	122	4.3	NS
Acetate (mmol/mol)	583	593	586	591	7.8	NS	470^{a}	501^{a}	552^{b}	567^{b}	11.1	*
Propionate (mmol/mol)	184	180	193	185	6.2	NS	278^{a}	228^{ab}	198^{b}	193^{b}	17.4	*
Acetate:Propionate	3.3	3.5	3.2	3.4	0.17	NS	1.8^{a}	2.3^{ab}	2.9^{b}	3.0^{b}	0.201	*
Butyrate (mmol/mol)	156	149	147	151	2.6	NS	170	185	182	177	7.3	NS
Valerate(mmol/mol)	77	78	75	73	2.8	NS	82^{a}	86^{a}	68^{b}	64^{b}	3.5	*
Plasma metabolites (mmol / l)												
Beta-hydroxybutyrate	0.41	0.38	0.33	0.41	0.037	NS	0.43	0.46	0.42	0.48	0.045	NS
Urea	4.14	4.10	3.94	3.97	0.144	NS	4.18	5.53	5.00	3.95	0.547	NS
Glucose	3.74	3.72	3.77	3.79	0.043	NS	3.78	3.73	3.61	3.35	0.213	NS

[1]RFS = Rapidly fermentable starch; [2]SFS = Slowly fermentable starch, [3]RFS+F = Rapidly fermentable starch + Fibre; F[4] = Fibre

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