Irish Journal of Agricultural and Food Research **48:** 57–73, 2009

Feed intake pattern, behaviour, rumen characteristics and blood metabolites of finishing beef steers offered total mixed rations constituted at feeding or ensiling

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Two experiments were undertaken. In Experiment 1, behaviour, intake pattern and blood metabolites, were recorded for steers offered total mixed rations (TMR) based on grass silage and concentrates, and constituted either at ensiling (E-TMR) or feed-out (F-TMR). Fourteen continental crossbred steers (mean starting weight 505 (s.d. 41.5) kg) were assigned to each of the following eight treatments: grass silage offered *ad libitum* (SO), E-TMR diets constituted in approximate dry matter (DM) ratios of grass:concentrates of 75:25 (EL), 50:50 (EM) and 25:75 (EH), F-TMR diets constituted in approximate DM ratios of grass silage:concentrates of 75:25 (FL), 50:50 (FM) and 25:75 (FH), and finally concentrates *ad libitum* (AL). Total DM intake increased linearly ($P < 0.001$) and the time spent eating and ruminating decreased linearly ($P < 0.001$) with increasing concentrate proportion. Animals on the F-TMR diets had higher total DM intakes ($P < 0.05$) and plasma glucose ($P < 0.05$) and urea ($P < 0.001$) concentrations than animals on the corresponding E-TMR diets. No effect of method of feed preparation on intake pattern or behaviour was recorded. In Experiment 2, four ruminally cannulated Holstein-Friesian steers of mean initial live weight 630 (s.d. 23.2) kg were used to evaluate rumen characteristics for four of the above diets (FL, EL, FH and EH) in a 4 × 4 latin square design. Higher concentrate diets resulted in lower rumen pH ($P < 0.05$), higher lactic acid ($P < 0.001$) and ammonia ($P < 0.05$) concentrations and lower acetate:propionate ($P < 0.05$). F-TMR was associated with a higher ($P < 0.05$) rumen volatile fatty acid concentration but no difference in other rumen fermentation characteristics compared to E-TMR. Concentrate proportion and

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method of feed preparation had no effect ($P > 0.05$) on rumen pool sizes but animals consuming the high concentrate diet had a faster ($P < 0.05$) rumen passage rate of NDF than animals on the low concentrate diet.

Keywords: beef cattle; behaviour; intake patterns; rumen characteristics; total mixed rations

Introduction

Grass silage is the primary component of beef finishing diets in Ireland and is generally supplemented with concentrates to overcome deficiencies in nutrient supply (McGee, 2005). Increasing the proportion of concentrates in the diet of finishing beef cattle increases total DM intake, live weight gain and carcass gain (Drennan and Keane, 1987; Caplis *et al*., 2005; Keane, Drennan and Moloney, 2006). The feeding of concentrates usually produces an increase in the propionate proportion and a decrease in the acetate proportion of the rumen volatile fatty acids (VFAs) along with an increase in lactate concentration (Van Soest, 1982). However, negative effects are often incurred when high levels of grain-based concentrates are fed, including decreased rumination resulting in inadequate salivary secretion, depressed rumen cellulolysis, and low pH values (Mould and Ørskov, 1983). These increase the risk of acidosis (Owens *et al.*, 1998).

In recent years, the feeding of complete diets or total mixed rations (TMR) to cattle has been introduced on many farms to mechanise feeding and save labour. A number of studies have reported increased performance from beef cattle (Cooke *et al*., 2004) and increased milk production from dairy cattle (Patterson and Mayne, 1997) when a TMR of grass silage and concentrates was fed compared with separate or twice daily feeding of concentrates. Co-ensiling grain with harvested grass and subsequently feeding the resultant TMR from the silo has also been shown to improve performance in beef (Nicholas and McLeod, 1966; Moore and Kennedy, 1994) and dairy cattle (Ferris and Mayne, 1994) compared with conventional feeding of grass silage with an equivalent amount of a cereal supplement. Kaufmann (1976) demonstrated that complete-diet feeding achieves a more constant rumen pH and fermentation pattern throughout the day than is seen with once or twice daily feeding of supplementary concentrates. This in turn induces higher cellulolytic activity, leading to a higher rate of passage and higher intake of roughage. Complete-diet feeding also facilitates feeding a higher level of concentrates without negatively affecting the acetate:propionate ratio in the rumen.

Cummins (2008) offered varying silage: concentrate ratio TMRs constituted at ensiling and reported lower intake and poorer performance and carcass traits than when similar TMRs constituted at feeding were offered. It was hypothesised that the lower intake of the TMRs constituted at ensiling may have been due to a larger volume and silage fermentation product concentration as a result of effluent absorption by the concentrate component and that this resulted in a slower clearance from the rumen and passage through the gastrointestinal tract. Therefore, the aim of this study was to investigate the effects, on intake pattern, behaviour, rumen characteristics and blood metabolites in steers, of (1) different dietary ratios of grass silage and concentrates, (2) method of feed preparation (TMR constituted at feeding or at ensiling). The diets in the present experiments were those used by Cummins (2008)

and the animals in Experiment 1 were part of the production experiment of Cummins (2008).

Materials and Methods

Feed preparation

The experimental silages used have been described by Cummins (2008). They were prepared from the first cut of a perennial ryegrass *(Lolium perenne)* sward, harvested (precision chop) on 7–9 June 2005, and ensiled without an additive. In three treatments, namely EL, EM and EH, a coarse concentrate ration was mixed with the herbage at ensiling (E-TMR) in approximate dry matter (DM) grass:concentrate ratios of 75:25, 50:50 and 25:75, respectively. A fourth treatment consisted of herbage ensiled alone (SO). The DM concentration of the grass was estimated regularly throughout the harvesting period drying a sub-sample in a microwave until a constant weight was achieved. These DM values were used to calculate the weight of grass DM in the loads to which the concentrate was to be added. The appropriate quantity of concentrate was then mixed with the grass on a concrete yard and the mix was pushed into the silo, spreading well to ensure thorough mixing of grass and concentrate. After filling the silos, the feedstuff was compacted, sealed with two sheets of black polythene (0.125 mm thickness; IS 246 1989) and weighted with tyres and silt. The concentrate ensiled with the herbage consisted of 847 kg rolled barley, 102 kg soyabean meal and 51 kg molasses per tonne. The mineral and vitamin pre-mix was omitted to avoid the risk of stimulating a clostridial fermentation.

Experiment 1 – Feed intake pattern, behaviour and blood metabolites

Animals and treatments: The animals used in this randomised complete block design study comprised 112 late maturing (80 Charolais and 32 Limousin) crossbred steers with a mean starting live weight of 503 kg. Prior to the experiment, these animals were housed in a slatted floor shed and offered grass silage *ad libitum* to standardise gastro-intestinal contents. Based on the mean of two consecutive daily live weights recorded at the start of the experiment, animals were blocked on weight, within breed type, and from within each block were randomly allocated to the following eight dietary treatments, all offered at *ad libitum* (percentage inclusions are on a DM basis):

1. Silage only (SO)
2. Silage (75%) plus concentrate (25%), offered as a TMR constituted at feeding (FL)
3. Silage (50%) plus concentrate (50%), offered as a TMR constituted at feeding (FM)
4. Silage (25%) plus concentrate (75%), offered as a TMR constituted at feeding (FH)
5. Silage (75%) plus concentrate (25%), offered as a TMR constituted at ensiling (EL)
6. Silage (50%) plus concentrate (50%), offered as a TMR constituted at ensiling (EM)
7. Silage (25%) plus concentrate (75%), offered as a TMR constituted at ensiling (EH)
8. Concentrate plus 1 kg silage DM $head^{-1}$ day^{-1} (AL)

The AL treatment was included to allow a complete assessment of the animal response to concentrate input from zero to *ad libitum*. Following allocation to treatment, all animals were treated with the anti-parasitic Qualimec (Janssen Animal Health; 1% ivermectin, 1% benzyl alcohol) and vaccinated against Infectious

Bovine Rhinotracheitis (IBR) and parainfluenza (PI3) using Bovilis (Intervet UK, Ltd.). They were also treated with Butox pour-on (Intervet Productions S.A.; deltamethrin 0.75% w/v) to control skin lice. They were subsequently housed in a slatted floor shed fitted with Calan-Broadbent electronic feeding doors (American Calan Inc., Northwood, NH, USA) to allow recording of individual feed intake.

Feeding: Silage was removed from each silo once daily using a shear grab. The FL, FM and FH diets (F-TMR), were constituted using SO and the same concentrate ration as that used in the E-TMR diets but with the addition of a mineral and vitamin premix. Each F-TMR diet was mixed daily for three minutes in an Abbey feeder wagon (Abbey Farm Machinery, Nenagh, Co. Tipperary) and individual allowances were weighed in to each animal. Animals on the FM, EM, FH, EH and AL treatments were adjusted gradually to their diets. All diets were offered at 1.1 times the previous day's consumption, intakes were recorded daily and refusals were discarded twice weekly. Animals had access to fresh water at all times. Animals on SO, EL, EM, and EH received 40, 40, 80 and 120 g head^{-1} day^{-1} of an appropriate mineral and vitamin premix dusted on the silage.

Experimental procedure: Feed intake pattern was recorded over a 24 h period on 56 animals on Day 86 (blocks 1 to 7 across all treatments) and on the remainder on Day 105 (blocks 8 to 14 across all treatments). Feed boxes were filled at 0830 and then weighed every 20 min for the first 2 h post-feeding, then every hour for the next 2 h and every 2 h thereafter to 12 h with a final weight at 24 h.

Animal behaviour, classified as either eating, ruminating or idling, was visually recorded every 15 min for 24 h for 56 animals on Day 107 (blocks 1 to 7) and for the remaining 56 animals on Day 157 (blocks 8 to 14). Occasions where animals were observed drinking water were omitted from the calculations as they contributed < 0.01 of total observations.

Blood metabolites

All 112 animals were blood sampled by jugular venipuncture for determination of glucose, β-hydroxybutyrate (βHB) and urea. To facilitate the work routine, 56 of the animals (blocks 1 to 7) were blood sampled on Day 100 and the remainder (blocks 8 to 14) were sampled on Day 155. Samples were obtained from each animal immediately before feeding (0830) and at 2, 6, 10 and 24 h after feeding using separate evacuated blood collection tubes containing lithium heparin and sodium fluoride as anticoagulants. All samples were centrifuged at 2000 × g for 15 min at 4 °C within 1 h of collection. Plasma was harvested, aliquoted and stored at –20 °C until assayed.

Experiment 2 – Rumen characteristics study

This study was undertaken at the same time as Experiment 1.

Animals and treatments: Four Holstein-Friesian steers of initial live weight 630 (s.d. 23.2) kg, each fitted with a 10 cm internal diameter rumen cannula (Bar Diamond, Inc., Parma, ID), were used to determine rumen characteristics for four of the experimental diets, namely FL, EL, FH and EH in a 2 (concentrate proportions) × 2 (methods of feed preparation) factorial arrangement of treatments. The experiment was a balanced 4 × 4 latin square design with four experimental periods of 26 days duration each. Following 20 days adaptation to the treatments, all measurements were undertaken during days 21 to 26 of each period. Animals were accommodated in

individual stalls and had access to water at all times. Feed was offered *ad libitum* during the adaptation phase and at 0.95 of *ad libitum* intake during the measurement phase. Fresh feed was offered once daily at 0830. Animals on EL and EH received 40 and 120 g head^{-1} day^{-1}, respectively, of a mineral and vitamin premix dusted on the silage. Feed was sampled daily at feeding from days 21 to 26. Samples were stored at –18 °C until the end of the experiment and were then pooled to give one composite sample per treatment per period for subsequent analysis.

Experimental procedure: On days 21 and 22 of each experimental period, rumen fluid samples (ca. 200 mL) were collected through the rumen cannulae (Moloney and Flynn, 1992) immediately prior to feeding and at 1, 2, 4, 6, 8, 12, 16 and 24 h post feeding to assess rumen fermentation characteristics. Rumen fluid pH was determined immediately after sampling using an Orion digital pH meter and glass electrode. A 20 mL sub-sample was then acidified with 0.5 mL of 9M sulphuric acid and stored at –18 °C for subsequent analysis.

The rumen pool sizes of liquid and solids were determined directly by manual removal of the total rumen contents. The liquid and solid fractions were weighed, sampled and contents were then returned to the rumen as described by Shiels (1998). Samples were stored at –18 °C for subsequent analysis. This procedure was conducted on Day 23 (0830), Day 24 (1130) and Day 25 (1530) of each period. These three times were chosen to represent the pre-feeding, completion of the main meal and full rumen conditions, respectively. On Day 26, two evacuations were conducted at 1030 and 1930 to estimate fractional clearance rate (K_{cl}). The animals did not receive any feed between the two evacuations, but water was freely available. Rumen contents were weighed and samples were taken and stored at –18 °C for subsequent analysis.

Chemical analyses

The DM concentration of the silages was determined by drying duplicate 200 g sub-samples at 85 °C for 16 h and then correcting for loss of volatiles using the equation of Porter and Murray (2001). A further sub-sample was dried at 40 °C for 48 h, ground through a hammer mill (Retsch GmbH & Co. K.G, Haan, Germany; 1 mm mesh sieve) and analysed for *in vitro* DM digestibility (DMD), ash, total N, neutral detergent fibre (NDF) and water soluble carbohydrates (WSC). The DMD values were determined by the method of Tilley and Terry (1963) with the modification that the final residue was isolated by filtration rather than centrifugation. Ash concentration was determined by complete combustion in a muffle furnace at 550 °C for 5 h. Total nitrogen (g/kg DM) was determined using the LECO FP-428 instrument based on the methods of the Association of Official Analytical Chemists (AOAC) 990-03 (1990). The WSC concentration was determined by the anthrone method (Thomas, 1977) and the NDF value exclusive of ash was determined using the Ankom fibre analyser (Van Soest, Robertson and Lewis, 1991). An aqueous extract was mechanically obtained from undried sub-samples of silage and used for measurement of pH (using an Orion SA720 pH meter and electrode), lactic acid (using the Olympus AU400 and the L-Lactic Acid UV-method test kit (Boehringer Mannheim/R-Biopharm catalogue number 10139084035)), volatile fatty acids (VFA) and ethanol (measured by gas chromatography (Ranfft, 1973)) and ammonia nitrogen (NH_3-N) (measured using the Olympus AU400 and

the Thermo Electron Infinity Ammonia Liquid Stable Reagent kinetic method).

Rumen fluid samples were centrifuged at 2000 g for 20 min. The supernatant was decanted and analysed for NH_3-N, lactic acid and volatile fatty acids (VFAs). The DM content of the rumen solid and liquid samples from the rumen evacuations were determined after drying at 60 °C for 48 h and subsequently corrected for volatile losses using the equation of Shiels *et al.* (1999). The solid samples were then ground through a hammer mill (1 mm mesh sieve) for analysis of ash and NDF.

Plasma βHB was analysed using an Olympus AU400 analyser and Randox laboratory kits (RB1008). Plasma urea and glucose were also measured using the Olympus AU400 analyser with testing kits from Olympus Diagnostics (OSR6134 and OSR6121, respectively).

Statistical analysis and calculations

All data were analysed using Statistical Analysis Systems (SAS, 2002–2003). For the behaviour measurements in Experiment 1, the animal was considered as the experimental unit because it was assumed that the behavioural traits (eating, ruminating, etc.) were dietary rather than socially mediated responses, and hence data were analysed using the general linear models procedure (Proc GLM) and are presented as the proportion of time animals were observed eating, ruminating and idling. Blood metabolites and feed intake pattern over 24 h were analysed using a repeated measures design (Proc MIXED). Treatment differences for both the behaviour and blood variables were evaluated using *a priori* contrasts for the linear, quadratic and cubic effects of silage:concentrate ratio, the effect of method of feed preparation (F-TMR vs. E-TMR) and its linear and quadratic interactions with silage: concentrate ratio.

In Experiment 2, feed intake and rumen characteristics were analysed using Proc GLM and a model appropriate for a latin square design with terms for animal, period, concentrate proportion, method of feed preparation and concentrate proportion × method of feed preparation interaction. The data are presented as the main effect means with the significance of factors and interactions indicated. Rumen digesta kinetics/clearance rates (K_{cl}) were calculated using the logarithmic transformation of the exponential equation $R_{1900} = R_{1100} \times e^{-K_{cl} \times t}$, where R_{1900} is the amount (kg) present at the second evacuation, R_{1100} is the amount (kg) present at the first evacuation, K_{cl} is the fractional clearance rate (%/h) and t is the time (h) between the two evacuations (Taweel *et al*., 2005).

Results

Experiment 1

Feed composition: The composition of the feed used for the feed intake pattern, behaviour and blood metabolite study is presented in Table 1. All feeds were well-preserved, as indicated by their low pH values, relatively low concentrations of NH_3-N and butyrate and high contribution of lactic acid to total fermentation products (FP).

Feed intake pattern and behaviour: Results for feed intake for the 24 h period when behaviour was monitored are shown in Table 2. Total DM intake increased with increasing concentrate proportion and the linear effect was significant ($P < 0.001$). There was an effect of method of feed preparation on total DM intake with animals on the F-TMR diets having a higher ($P < 0.05$) total DM intake than those on the E-TMR diets. No concentrate proportion × method of feed preparation interaction was evident for total DM intake over the 24 h period.

Table 1. Chemical composition of the feeds offered in Experiment 1 (mean (s.d.))

	Feeds[1]				
	SO	EL	EM	EH	Concentrate
Chemical composition at feed-out					
Dry matter (DM) (g/kg)	234 (5.0)	266 (20.9)	333 (22.5)	436 (26.5)	861 (5.2)
pH	3.9 (0.15)	3.9 (0.08)	4.0 (0.08)	4.1 (0.04)	
in vitro DM digestibility (g/kg)	721 (11.8)	775 (15.2)	796 (9.2)	820 (13.0)	886 (6.1)
Starch (g/kg DM)					464 (14.3)
Ash (g/kg DM)	80 (5.6)	72 (6.3)	66 (3.2)	61 (4.6)	43 (6.3)
Total N (g/kg DM)	22 (1.5)	24 (1.9)	25 (1.1)	26 (1.2)	25 (0.6)
Neutral detergent fibre (g/kg DM)	545 (33.5)	431 (34.3)	396 (36.0)	317 (41.8)	184 (11.1)
WSC (g/kg DM)	16 (8.7)	14 (3.1)	21 (10.3)	28 (10.9)	
Estimated net energy[2] (UFV/kg)	0.77	0.86	0.96	1.05	1.12
Fermentation characteristics (g/kg volatile-corrected DM, unless otherwise stated)					
Ethanol	18 (3.8)	23 (9.0)	10 (2.9)	10 (4.9)	
D-Lactate	74 (16.5)	58 (12.7)	43 (12.5)	33 (13.9)	
L-Lactate	85 (19.6)	71 (7.7)	54 (10.7)	46 (8.4)	
Acetic acid	32 (13.6)	15 (3.6)	18 (5.5)	13 (2.9)	
Propionic acid	2.2 (2.00)	0.3 (0.24)	1.0 (0.55)	0.6 (0.28)	
Butyric acid	0.3 (0.80)	0.1 (0.12)	0.3 (0.42)	0.5 (0.42)	
Fermentation products[3] (FP) (g/kg DM)	211 (28.1)	168 (25.8)	127 (25.6)	101 (40.8)	
Ammonia nitrogen (g/kg total N)	94 (24.1)	81 (21.6)	79 (7.0)	55 (17.9)	

[1] SO = silage only; EL, EM and EH = Total mixed ration constituted at ensiling (E-TMR) in approximate DM ratios of grass: concentrates of 75:25, 50:50 and 25:75, respectively.
[2] Unité Fourragère Viande (Jarrige, 1989).
[3] FP = lactic acid + volatile fatty acids + ethanol.

Table 2. Feed intake and behaviour of animals in Experiment 1

	Treatment[1]								s.e.	Significance[3]		
	SO	FL	FM	FH	EL	EM	EH	AL		L	Q	M
Total dry matter (DM) intake (kg)	6.25	9.21	9.43	11.18	6.60	7.22	10.11	11.46	0.392	***		*
Behaviour[4]												
Eating	0.16	0.13	0.09	0.07	0.12	0.11	0.07	0.04	0.024	***		
Ruminating	0.41	0.35	0.28	0.23	0.34	0.29	0.22	0.16	0.016	***		

[1] SO = silage only; FL, FM and FH = TMR constituted at feeding (F-TMR) in approximate DM ratios of grass silage: concentrates of 75:25, 50:50 and 25:75; EL, EM and EH = E-TMR (see footnote Table 1); AL = concentrate *ad libitum* with 1 kg silage DM.
[2] For n=14.
[3] L, Q = linear, quadratic effects of increasing concentrate proportion; M = E-TMR vs. F-TMR.
[4] Proportion of time.
There were no significant concentrate proportion × method of feed preparation interactions.

Results for behaviour are also shown in Table 2. The proportion of time animals spent eating and ruminating decreased linearly ($P < 0.001$), while the proportion of time spent idling increased linearly ($P < 0.001$), with increasing concentrate proportion. Method of feed preparation had no significant effect on behaviour.

The pattern of DM intake over 24 h is shown in Figure 1. All animals had higher ($P < 0.05$) intake in the first 20 min than in any subsequent period of the first 2 h.

Animals on the AL treatment consumed 0.35 of their total 24 h intake in the first 20 min and had higher ($P < 0.05$) intake than all other treatments for the first hour post-feeding. During the subsequent 4 h period, animals on the AL consumed relatively little feed, and then increased intake again to 8 h, by which time they had eaten 0.74 of their total daily intake. In contrast, animals on the SO treatment displayed a more even pattern of intake, consuming only 0.12 of their total 24 h intake in the first 20 min. They consumed 0.43 of their intake in 4 h and had consumed 0.67 of their total daily intake by 8 h. Animals on SO consumed very little feed overnight (0.08 of total DM between 12 and 24 h). The intake patterns for FL, FM, FH, EL, EM and EH were intermediate to those of AL and SO with animals on the higher concentrate diets generally having greater intakes in the first hour and greater total intakes than those on the lower concentrate diets. Animals offered E-TMR and F-TMR diets had similar ($P > 0.05$) intake patterns at the same forage to concentrate ratios. Feed intake was relatively low overnight for all treatments (0.22 of total DM was consumed between 12 and 24 h).

Blood metabolites: Plasma glucose concentration increased linearly ($P < 0.05$), while urea concentration increased both linearly ($P < 0.001$) and quadratically ($P < 0.001$), with increasing concentrate proportion (Table 3). There was a quadratic ($P < 0.001$) relationship between βHB concentration and concentrate proportion. Method of feed preparation had a significant effect on both plasma glucose and urea concentrations with animals on the F-TMR diets having higher concentrations of both than animals on the E-TMR diets. Sampling time and treatment × sampling time interactions were significant for all blood parameters and these interactions for glucose, βHB and urea are presented in Figures 2a, b and c, respectively. Highest glucose concentration was generally observed prior to feeding. Glucose concentration declined over the first 2 h, then increased over the following 4 h (except for SO), declined again over the next 4 h and then increased to their normal pre-feeding values over the remaining 14 h. At 2, 6 and 10 h post-feeding FM and FH had higher ($P < 0.05$) glucose concentrations than EM and EH respectively, while FL and

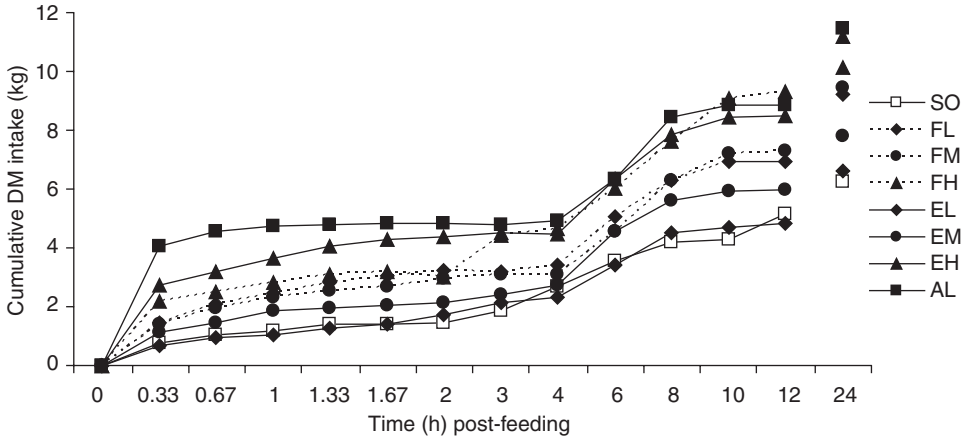

Figure 1. Cumulative total dry matter (DM) intake (kg) for 24 h after feeding in Experiment 1.

Table 3. Blood metabolites for Experiment 1

Blood metabolite (mmol/L)	Treatment[1]								s.e.[2]	Significance[3]				
	SO	FL	FM	FH	EL	EM	EH	AL		Time	Treatment × Time	L	Q	M
Glucose	4.10	4.36	4.53	4.56	4.19	4.26	4.32	4.44	0.125	***	**	*		*
β-hydroxybutyrate	0.21	0.30	0.34	0.30	0.26	0.36	0.40	0.22	0.019	***	***		***	
Urea	3.52	3.66	4.18	4.71	3.31	3.92	3.76	5.47	0.149	***	***	***	***	***

[1, 2, 3] See footnotes to Table 2.
There were no significant concentrate proportion × method of feed preparation interactions.

EL had similar glucose concentrations at each sampling time-point.

As method of feed preparation had no significant effect on βHB concentration, the values for the F-TMR and E-TMR treatment means were combined for the low, medium and high concentrate proportions (Figure 2b). There was no significant difference at any time between AL and SO. Concentration of βHB increased ($P < 0.001$) for all treatments post-feeding and thereafter remained relatively stable until 10 h post-feeding. Subsequently, βHB concentration decreased ($P < 0.01$) for all treatments and returned to their original level after 24 h.

Plasma urea concentration was lowest for SO and highest for AL before feeding (Figure 2c). Thereafter to 10 h, the urea concentration for SO increased and AL decreased before returning to their original pre-feeding values by 24 h. For the other treatments, plasma urea concentration increased over the first 2 h and then remained relatively stable to 10 h before returning to their original pre-feeding values by 24 h.

Experiment 2: Rumen characteristics

Silage composition: The chemical composition of the four diets used in this experiment is presented in Table 4. All feeds were well-preserved as indicated by their low pH values (3.9 to 4.1), high lactic acid: acetic acid ratio (8.1 to 9.8:1), low ammonia nitrogen values (38 to 72 g/kg N) and negligible propionic and butyric acid concentrations (0.2–0.7 g/kg DM). The F-TMR diets had a numerically higher DM and WSC concentration and a lower DM digestibility and ash concentration than the corresponding E-TMR diets. Both F-TMR and E-TMR diets had a similar total N concentration. Total FP and ammonia nitrogen were numerically higher for FL than FH and for EL than EH.

Feed intake: Feed intakes (restricted to 0.95 of previous *ad libitum* intake) are presented in Table 5. Increasing the concentrate proportion in the diet increased ($P < 0.01$) DM intake, and feeding diets mixed at feed out led to a higher ($P < 0.05$) intake than for those mixed at ensiling.

Rumen fermentation: Results for rumen fermentation variables are also shown in Table 4. Animals offered the high concentrate-proportion diets had a lower ($P < 0.05$) rumen pH than those offered the low concentrate proportion diets while method of feed preparation had no significant effect on rumen pH. Concentrate proportion had an effect on rumen lactic acid and ammonia concentrations with animals offered the high concentrate-proportion diets having a higher ($P < 0.001$) lactic acid concentration and a higher ($P < 0.05$) ammonia concentration than those offered the low concentrate-proportion diets. Animals on the F-TMR diets had higher ($P < 0.05$) total VFA values than those offered the E-TMR

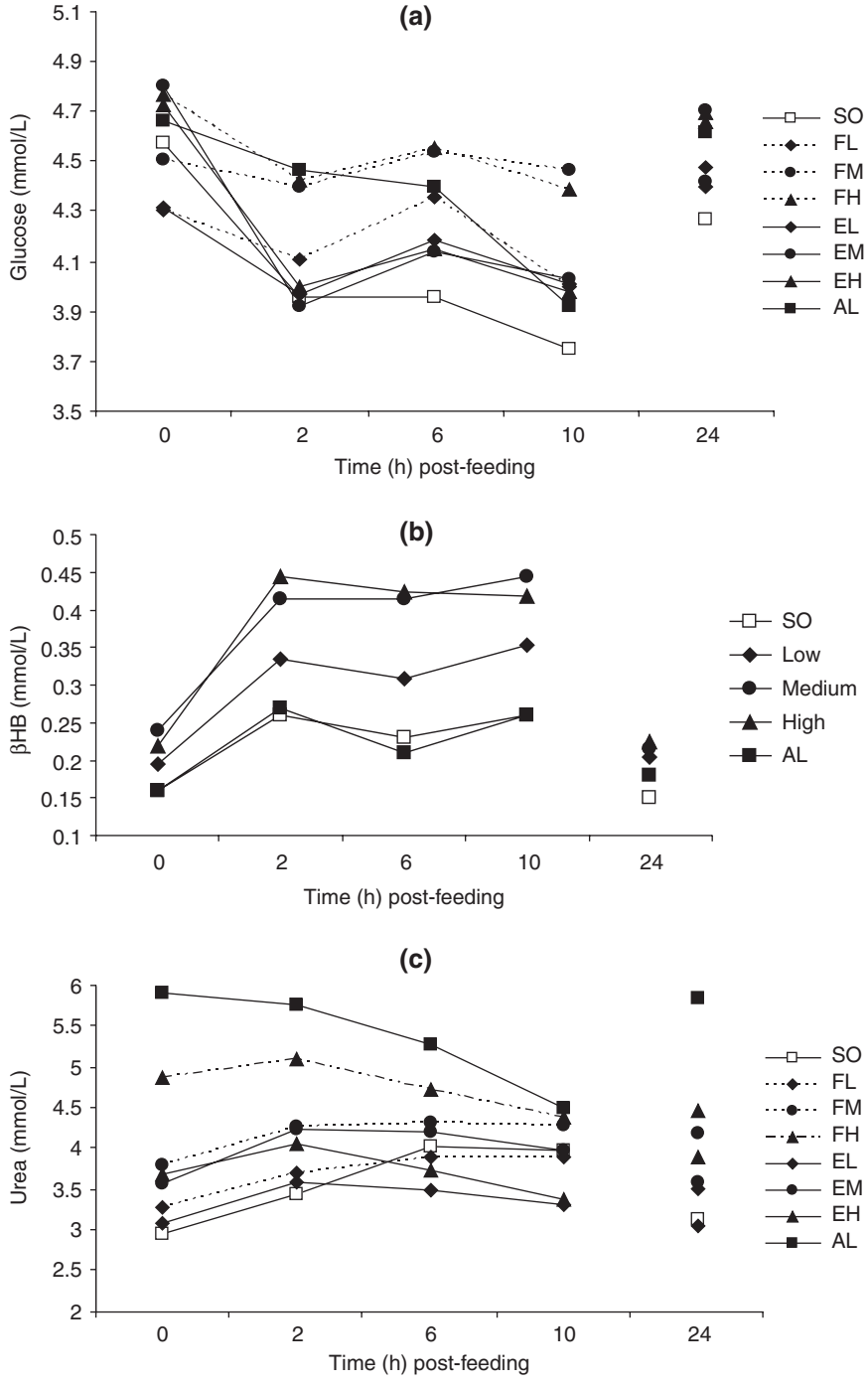

Figure 2. Effects of treatment and sampling time on plasma (a) glucose, (b) β-hydroxybutyrate (βHB) and (c) urea concentrations (mmol/L) in Experiment 1.

Table 4. Chemical composition of the feeds (mean (s.d.)) offered in Experiment 2

	Feeds[1]			
	FL	FH	EL	EH
Chemical composition at feed-out				
Dry matter (DM) (g/kg)	279 (5.2)	452 (6.7)	260 (18.4)	432 (20.5)
pH	3.9 (0.05)	4.1 (0.08)	3.9 (0.08)	4.1 (0.04)
in vitro DM digestibility (g/kg)	759 (10.5)	791 (17.3)	770 (12.2)	805 (13.0)
Ash (g/kg DM)	74 (6.2)	68 (4.2)	66 (4.5)	60 (4.1)
Total N (g/kg DM)	23 (0.5)	24 (0.9)	24 (1.3)	25 (1.2)
Neutral detergent fibre (g/kg DM)	472 (7.3)	376 (25.2)	424 (24.2)	310 (21.8)
WSC (g/kg DM)	24 (6.7)	45 (4.1)	16 (2.6)	29 (3.9)
Fermentation characteristics (g/kg volatile-corrected DM, unless otherwise stated)				
Ethanol	11 (2.3)	6 (0.9)	18 (7.6)	8 (2.9)
D-Lactate	54 (7.4)	25 (2.5)	60 (9.1)	39 (10.4)
L-Lactate	60 (8.2)	36 (4.9)	73 (5.2)	49 (11.8)
Acetic acid	14 (2.6)	7 (1.9)	15 (1.6)	9 (2.9)
Propionic acid	0.3 (0.12)	0.4 (0.17)	0.3 (0.28)	0.5 (0.42)
Butyric acid	0.7 (0.42)	0.2 (0.17)	0.1 (0.15)	0.3 (0.12)
Fermentation products[2] (FP) (g/kg DM)	140 (26.7)	75 (14.7)	166 (31.2)	106 (21.0)
Ammonia nitrogen (g/kg total N)	72 (10.2)	38 (11.6)	79 (13.2)	43 (9.3)

[1] See footnote to Table 2.
[2] Lactic acid + volatile fatty acids + ethanol.

Table 5. Feed intake and the pH, concentrations of ammonia, lactic acid and total volatile fatty acids (VFAs) and molar proportions of individual VFAs in the rumen fluid in Experiment 2

	Concentrate proportion (C)		Method of feeding (M)		s.e.[1]	Significance	
	Low	High	F-TMR	E-TMR		C	M
Total dry matter (DM) intake[2] (kg/day)	9.33	13.49	12.56	10.27	0.576	**	*
Rumen fermentation variables							
pH	6.41	6.17	6.32	6.26	0.056	*	
Lactic acid (mg/L)	109	163	136	135	4.1	***	
Ammonia (mg/L)	103	155	141	117	10.4	*	
Total VFA (mmol/L)	107	112	119	100	5.0		*
Molar proportions (mmol/mol VFA)							
Acetic acid	562	482	522	522	16.1	*	
Propionic acid	203	246	235	214	13.2	P = 0.06	
Iso-butyric acid	14	14	14	14	0.5		
N-butyric acid	137	157	136	158	10.1		
Total butyric acid	151	171	150	172	9.9		
Iso-valeric acid	54	52	52	54	4.0		
N-valeric acid	30	51	42	39	5.2	*	
Total valeric acid	84	103	94	93	6.7	P = 0.09	
Acetate:Propionate ratio	2.8	2.1	2.4	2.5	0.17	*	
NGR[3]	4.3	3.5	3.7	4.2	0.34		

[1] For n=8.
[2] Restricted to 95% of prior *ad libitum* intake.
[3] Non-glucogenic ratio ((acetic acid + (2*butyric acid))/propionic acid).
There were no significant concentrate proportion × method of feed preparation interactions.

diets while individual molar VFA proportions were unaffected by method of feed preparation. Animals offered the high concentrate-proportion diets had a lower ($P < 0.05$) molar proportion of acetic acid and a higher ($P = 0.06$) molar proportion of propionic acid than animals offered the low concentrate-proportion diets. The molar proportion of *n*-valeric acid was higher ($P < 0.05$) in animals on the high concentrate-proportion diets than those on the low concentrate-proportion diets while the molar proportion for total valeric acid tended towards significance ($P = 0.09$) with animals on the high concentrate-proportion diets having the higher values. The acetate:propionate ratio was lower ($P < 0.05$) for animals on the high concentrate-proportion diets than for those on the low concentrate-proportion diets.

Rumen pool size: Rumen pool sizes are presented in Table 6. No effect of concentrate proportion or method of feed preparation was found for rumen liquid, DM, OM or NDF pool sizes.

Fractional clearance rate: Neither concentrate proportion nor method of feed preparation had an effect on the fractional clearance rates of DM or OM (Table 6). Fractional clearance rate of NDF was higher ($P < 0.05$) for animals offered diets with the higher concentrate proportion.

Discussion

The purpose of these studies was to describe the feed intake pattern, eating and ruminating behaviour, rumen characteristics and blood metabolites of finishing beef steers offered different ratios of grass silage and concentrates as a TMR constituted at ensiling or at feeding and to ascertain if there were interactions between supplementary concentrate proportion and method of feed preparation. All feeds were well preserved (extensive lactic acid dominant fermentation) of moderately high digestibility (721 g/kg *in vitro* DMD for SO). The long-term intake, performance and carcass traits of these steers have been described previously by Cummins (2008). Live-weight gains for animals offered SO, FL, FM, FH, EL, EM, EH and AL were 158, 742, 867, 994, 565, 732, 779 and 938 (s.e. 64.0) g/day. Corresponding carcass-weight gains were 146, 479, 623, 682, 417, 513, 589 and 695 (s.e. 32.9) g/day. Increasing the concentrate proportion in the diet increased intake and weight gain while offering the F-TMR diets increased total DM intake, live weight gain and carcass gain, compared to the corresponding E-TMR diets.

Feed intake pattern: The linear relationship observed between concentrate proportion and total DM intake agrees with Lardy *et al.* (2004) who found a linear increase in total DM intake when grass hay was supplemented with increasing levels of barley. Despite the continuous availability of feed, DM intake was not equally distributed during the 24 h after feeding. These diurnal peaks of eating activity agree with the findings of Cozzi and Gottardo (2005) who observed similar behaviour for housed Limousin bulls offered a TMR *ad libitum* once daily in the morning.

Eating and ruminating behaviour: The observed reduction in eating and ruminating times as the forage: concentrate ratio in the diet decreased is in agreement with findings from other studies (Bines and Davey, 1970; Welch and Smith, 1971; Thorp *et al*., 1999) with the lower fibre or cell wall constituents being the main contributing factor to reduced rumination of animals on high concentrate diets (Welch and Smith, 1970).

Table 6. Mean rumen pool sizes and fractional clearance rates in Experiment 2

	Concentrate proportion (C)		Method of feeding (M)		s.e.[1]	Significance[2]	
	Low	High	F-TMR	E-TMR		C	M
Rumen pool size (kg)							
Liquid (fresh)	73.2	67.6	72.0	68.9	3.70		
Dry matter	9.8	10.9	11.0	9.7	0.61		
Organic matter	9.0	10.0	10.1	8.9	0.57		
Neutral detergent fibre	7.0	6.4	6.5	6.9	0.39		
Fractional clearance (K_{cl}) rate (%/h) for							
Dry matter	3.3	5.0	4.7	3.6	0.69		
Organic matter	4.8	7.3	6.9	5.2	1.0		
Neutral detergent fibre	2.1	4.5	3.7	2.9	0.52	*	

[1,2] See footnotes to Table 5.
There were no significant concentrate proportion × method of feed preparation interactions.

Rumen fermentation: The lower rumen pH observed in animals on the higher proportion of concentrates agrees with many previous findings (Mould, Ørskov and Mann, 1983; Carro *et al.*, 2000; Sutton *et al*., 2003) and is primarily due to the increased production of VFA and lactic acid as a result of rapid fermentation of readily fermentable energy sources such as barley grain (Obara, Dellow and Nolan, 1991). It may also have been associated with reduced saliva production. The higher ruminal lactate concentration associated with the higher concentrate proportion agrees with results from Waldo and Schultz (1956) and Ghorban, Knox and Ward (1966), but as pH values did not fall below 5.6 (the threshold for chronic acidosis), the lactate-fermenting bacteria were still able to function (Owens *et al*., 1998).

The higher rumen ammonia concentration evident for animals in the higher concentrate proportion reflects the corresponding higher intake of nitrogen. It may also indicate a possible increase in the amount of protozoa in the rumen which has been associated with increased recycling of rumen nitrogen (Chamberlain *et al*., 1985). Carro *et al*. (2000) observed a similar trend in ruminal ammonia concentration when the concentrate proportion of an alfalfa hay based diet was increased. On all diets, concentration of ammonia was above the minimum level considered adequate for maximal microbial growth rate (50 mg/L; Satter and Slyter, 1974).

While total VFA concentration was unaffected by concentrate proportion, the pattern of rumen fermentation changed from a high acetate concentration on the diets with a low concentrate proportion to a high propionate concentration on the high concentrate-proportion diets. It is well established that forage-based diets encourage the growth of acetate producing bacteria at the expense of propionate fermenters, while starch-rich diets favour the development of propionate-producing bacteria, thus leading to a higher molar proportion of propionic acid on starchy concentrate diets (France and Dijkstra, 2005). The marked increase in the proportion of butyric acid in rumen VFA for the high concentrate-proportion diets, although not statistically significant, is in agreement with other published reports including those of Thorp *et al.* (1999), Carro *et al*. (2000) and Lardy *et al*. (2004). According to Williams and Coleman (1997), this increase in butyrate occurs

under conditions where concentrate diets encourage the development of a large protozoal population. The higher valeric acid concentration observed in animals on the diets with a high concentrate proportion agrees with the report of Sutton *et al*. (2003). The lower acetic:propionic ratio on the high concentrate diets reflects the change in rumen fermentation as a result of the change in diet from a predominantly forage based diet to a concentrate-based diet. It is well known that pH in the rumen influences the acetic:propionic ratio (Esdale and Satter, 1972; Kaufmann, 1976), with a low pH associated with a decreased acetic:propionic acid ratio, as was evident in this experiment. The higher total VFA concentration for animals offered the F-TMR compared with those on the E-TMR diets was most likely driven by the higher intake of animals on the F-TMR diets. Despite higher plasma glucose concentrations being observed for the F-TMR diets (Experiment 1), rumen propionate, the main gluconeogenic pre-cursor in ruminants (Donkin and Armentano, 1995), did not differ between F-TMR and E-TMR. Therefore, another precursor such as rumen lactate (Brockman, 2005) may have contributed to the higher plasma glucose concentration for the F-TMR diets.

Rumen pool sizes and fractional clearance rate: The absence of an effect of concentrate proportion on rumen liquid, DM, OM and NDF pools agrees with the findings of Poore, Moore and Swingle (1990) who reported similar rumen liquid and DM pools in steers fed increasing levels of concentrates. According to Owens and Goetch (1988), greater amounts of rumen liquid may be associated with coarse roughage diets than with diets of concentrate or processed roughage. This is supported by the present findings in that a numerically greater liquid pool was observed in animals on the diets with a low concentrate proportion. A numerically higher NDF pool was also observed for the low concentrate-proportion diets reflecting their higher fibre concentration. Despite the higher intake of the animals on the high concentrate diets and on the F-TMR diets, DM and OM pool sizes did not increase correspondingly. This may perhaps be explained by the numerically higher clearance rates of DM and OM observed for these diets.

The significantly higher clearance rate of NDF and the numerically higher clearance rates of DM and OM for the high concentrate-proportion diets were possibly due to the higher intake and smaller particle size of these diets (Van Soest, 1982). The numerically lower clearance rate in animals on the E-TMR diets could at least partially explain their lower intakes.

Blood metabolites: The linear increase in plasma glucose concentration reflect the increased DM intake with increasing concentrate proportion while the higher glucose concentration evident in animals on F-TMR than on E-TMR diets reflect their higher intake. The immediate drop in glucose levels after feeding is in agreement with previous reports (Thye, Warner and Miller, 1970; Moloney *et al*., 1994; Shiels, 1998). Most of the butyrate produced in the rumen is extensively metabolized to the ketone bodies, acetoacetate and βHB, by epithelial cells (Bergman, 1990). Thus, the higher plasma βHB concentration associated with increased concentrate proportions in treatments FM, EM and EH were consistent with the numerically higher rumen butyrate concentrations generated by the high concentrate-proportion diets (Experiment 2).

The increase in plasma urea concentration with increasing concentrate proportion

agrees with Oltjen *et al*. (1967), reflecting increased crude protein intake. The increased plasma urea concentration followed from the increase in rumen ammonia concentration with increasing concentrate proportion in the diets. Similarly, the higher DM intake and thus higher CP intake for F-TMR than for E-TMR diets were reflected in higher plasma urea concentrations.

Conclusions

It is concluded that increasing the concentrate proportion in the diet increased total DM intake despite the decreased proportion of time spent eating and ruminating. It also changed the rumen fermentation pattern from a cellulolytic to an amylolytic profile. Feeding varying silage:concentrate ratio TMRs constituted at feeding resulted in greater intake, higher total VFA concentration and higher plasma glucose and urea concentrations compared with feeding similar TMRs constituted at ensiling. While the data fail to provide a clear explanation for the lower intake and hence lower performance on the E-TMR diets in the beef production study reported by Cummins (2008), the most likely reason was a slower clearance rate of DM and its components from the rumen. For DM, OM and NDF, the fractional rumen clearance rates on E-TMR were proportionately only 0.76, 0.75 and 0.78, respectively, of those for F-TMR.

Acknowledgements

The authors acknowledge Mr. J.A. Farrell for his skilled technical assistance, the Grange farm staff in particular Mr. B. Duffy, Mr. P. Reilly and Mr. G. Costello for care of and assistance with the animals and the staff of Grange Laboratories for chemical analysis. Also, acknowledgement is made to Dr. M. McGee and Dr. H.Z. Taweel for their help and expertise. B. Cummins was in receipt of a Teagasc Walsh Fellowship.

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Received 20 December 2007