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Effect of Supplementing Grass Silage-Based Diets with Concentrate Carbohydrate Sources with Different Fermentation Profiles on N Metabolism of Beef Heifers Fed to Maintenance

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Abstract: The synchronous supply of energy and nitrogen (N) substrates to the rumen microbes on grass silage (GS)-based diets can potentially lead to reduced levels of N excreted in the urine. The objective of this study was to evaluate the effect of supplementing GS-based diet with carbohydrate sources differing in rumen fermentation profile on N metabolism of beef heifers. Six Belgian Blue × Holstein Friesian cross beef heifers (487 ± 29 kg BW) were used in a 3 × 3 Latin Square design (n = 6). Dietary treatments were: (RB) GS supplemented with rolled barley; (MM) GS supplemented with maize meal and; (SH) GS supplemented with soya hulls offered at 40:60 forage to concentrate ratio on a dry matter (DM) basis, at maintenance feeding (40 g DM/kg $BW^{0.75}$). Carbohydrate source had no effect on DM, organic matter, or N intake or total N excretion and the amount of N excreted in the urine ($p > 0.05$). Animals offered MM excreted a higher percentage of N in the faeces and a lower percentage of N in the urine compared to animals offered RB ($p < 0.05$). There was a time by interaction for ruminal ammonia (NH_3) concentrations ($p < 0.01$). Ruminal NH_3 concentrations peaked at 2 h post-feeding for all treatments. At 3 h post-feeding, ruminal NH_3 concentrations for the RB treatment remained higher compared to MM and SH treatments. Molar proportions and total ruminal volatile fatty acids were similar among dietary treatments ($p > 0.05$). Supplementing GS-based diets with different carbohydrate sources had no impact on the total level of N excreted or the amount of N excreted in the urine. However, there was a higher percentage of N excreted in the faeces and a lower percentage of N excreted in the urine when animals were offered MM compared to those offered RB ($p < 0.05$).

Keywords: beef cattle; carbohydrates; crude protein; nitrogen balance; nitrogen excretion; ruminal fermentation

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1. Introduction

Globally, there are growing concerns as levels of ammonia (NH_3) in the atmosphere continue to rise [1]. Excess nitrogen (N) excreted from agriculture contribute to atmospheric NH_3 [2,3] responsible for 93% of total NH_3 emissions within the European Union (EU) [4]. When redeposited, ammonia increases acidification and the eutrophication of terrestrial and aquatic ecosystems [5]. Furthermore, NH_3 reacts with atmospheric acids to form secondary particles (particulate matter, $PM_{2.5}$), which contribute to air pollution, estimated to be responsible for 4.2 million premature deaths worldwide in 2016 [6]. In Ireland, agriculture accounts for 99% of total NH_3 emissions, with the cattle sector responsible for 90% of this total, owing to animal housing/storage and land spreading of manures accounting for 47.1%, and deposition at grazing accounting for 12.3% [7].

Beef cattle are inefficient in utilising N, only retaining 10–20% of N consumed [8], resulting in large amounts of ingested N being excreted in urine and faeces. Reducing urinary N excretion is more favourable, as the rate of volatilisation of urinary urea N to NH_3 is much faster compared to the organic N compounds in faeces [9,10]. This problem is particularly relevant to Ireland, where the main livestock production systems are pasture based, with limited supplementary feeding for much of the year [11]. Typically, in an Irish suckler calf to beef system, pasture, grass silage (GS) and concentrates make up 66%, 27% and 7%, respectively, of feed dry matter intake (DMI) annually [12], with barley as the traditional carbohydrate source [13].

Grass silage is the main conserved forage fed to beef cattle in Ireland. During the ensiling process, water-soluble carbohydrates are the primary fermentation substrate and plant proteins are broken down to amino acids and $NH_{3,}$ the extent of which is dependent on the rate of pH decline [14]. Therefore, the main carbohydrate substrates available for fermentation in the rumen are slowly fermented fibre substrates, cellulose and hemicellulose, while the N compounds in GS are mainly soluble, leading to instant degradation within the rumen [15]. This asynchronous release of energy and N components in the rumen has been considered an important cause of the low N use efficiency for microbial growth observed with diets such as GS [16]. The incorporation of cereal grains in concentrate feed formulations can provide an energy source in the form of starch to the rumen microbes, thus allowing a greater capture of N in the rumen [17].

Globally, 36% of cereal grains are used for livestock feed [18]; however, the inclusion of by-products in livestock feeds is increasing in Ireland, with imports of maize and soya hulls increasing from 925,000 to 1,110,000 tonnes, and 350,000 to 400,000 tonnes, respectively, between 2015 and 2017 [19]. The starch found in wheat, oats, and barley is more rumen degradable than the starch in maize [20]. Castillo et al. [21] observed that when maize starch replaced barley starch in the diet, there was an improvement in the portion of ingested N recovered in the faeces and a reduction in the portion of N excreted in the urine, suggesting that circulating urea N was rerouted into the large intestine to support increased microbial protein synthesis in the caecum [22].

Soya hulls contain a variety of energy substrates for ruminal microbes, including non-fibre carbohydrates and a highly digestible neutral detergent fraction (NDF) [23]. Contrasting results have been found in the ruminal NH_3 concentration when soya hulls replaced grains in the diets of dairy cows [24]. However, when soya hulls replaced barley as the energy source in the concentrate offered to growing cattle fed grass silage, performance parameters were not affected [25].

It was hypothesised that offering a carbohydrate that is rapidly degraded within the rumen will in turn capture more N within the rumen and reduce N excretion.

Therefore, the objective of this study was to evaluate the effect of supplementing grass silage-based diets with concentrate carbohydrate sources with different fermentation profiles on N metabolism of beef heifers fed to maintenance.

2. Materials and Methods

This experiment was conducted at UCD Lyons Research Farm, Celbridge, Naas, Co. Kildare, Ireland, W23 ENY2 (53°17′56″ N, 6°32′18″ W). All experimental procedures involving use of animals were approved by the Animal Research Ethics Committee (AREC) at University College Dublin (UCD) and managed and cared for according to the European directive 2010/63/EU and S.I. No. 543 of 2012, under license from the Health Products Regulatory Authority (HPRA) (approval number: AE18982/P083).

2.1. Experimental Design and Dietary Treatments

Six beef heifers (*Bos taurus* strain Belgian Blue × Holstein Friesian) with an initial body weight of 487 ± 29 kg, were surgically fitted with permanent ruminal cannula (100 mm i.d.) (Bar Diamond Inc. Idaho, USA) and assigned to one of three dietary treatments in a replicated 3 × 3 Latin Square design (*n* = 6). Dietary treatments were as follows: RB) GS supplemented with rolled barley; MM) GS supplemented with maize meal; and SH) GS supplemented with soya hulls offered at 40:60 forage concentrate ratio on a dry matter

(DM) basis. All diets were formulated to be isonitrogenous and balanced with soya bean meal (Table 1). Diets were offered at maintenance (40 g DM/kg $BW^{0.75}$) [26] twice daily as a total mixed ration (TMR) at 08:00 and 16:00 h using a Calan Data Ranger (American Calan, Northwood, New Hampshire, USA). The GS used during this experiment consisted of predominantly perennial ryegrass (*Lolium perenne* L.). The crop was felled during the early booth stage of vegetation (growth stage 410; [27], wilted for 16 h, baled, and wrapped using a McHale Fusion 3 Integrated baler/wrapper (McHale, Ballinrobe, Co. Mayo, Ireland). The crop was ensiled without the use of an additive.

Table 1. Ingredient composition and chemical composition of dietary treatments.

Ingredient Composition (kg DM^{-1})	DIET		
	RB	MM	SH
Rolled barley	3.0	-	-
Maize meal	-	3.0	-
Soya hulls	-	-	3.0
Soya bean meal	0.77	0.94	0.77
Grass silage	1.47	1.47	1.47
Barley straw	1.0	1.0	1.0
Mineral premix	0.10	0.10	0.10
Chemical composition (g kg DM^{-1})			
Dry matter (g kg^{-1})	44.72	44.12	44.01
Crude protein	13.45	13.33	13.62
Starch	17.14	19.09	0.67
Neutral detergent fibre	30.35	28.99	49.63
Acid detergent fibre	16.85	16.36	32.49
Ash	6.37	6.70	7.15
Ether extract	1.77	1.44	0.87
Gross energy (MJ/kg DM)	15.22	15.31	15.14

Each experimental period consisted of a 14 d dietary adjustment period, where the animals were fed their respective diets using a Calan Broadbent controlled feeding system (American Calan, Northwood, New Hampshire, USA), followed by an 11 d experimental period, where the animals were housed in metabolism stalls (1.4 × 1.8 m). During this period in the metabolism stalls, animals were allocated the first 3 d for acclimatisation, followed by 8 d to facilitate a N-balance study, rumen sample collection and in sacco DM degradability determination. While in the metabolism house, each animal was assigned to their own individual stall for the duration of the experiment with *ad libitum* access to water.

2.2. Data and Sample Collection

During the N-balance study, all animals were fitted with a specially constructed harness system to facilitate the separate collection of urine and faeces as previously described in Kirwan et al. [28].

Samples of concentrates (rolled barley, maize meal, soya hulls and soya bean meal) were collected weekly, while GS and TMR samples were collected daily, later pooled per treatment and per animal for each experimental period. Samples were dried at 55 °C for 48 h for chemical analysis with additional samples frozen and stored at −20 °C for later total N analysis. Faecal and urine samples collected during the N-balance study were prepared as previously described in Whelan et al. [29].

On d 1 and 5 of each N-balance period, blood samples were collected by jugular venepuncture at 1600 h prior to pm feeding into blood collection tubes containing Lithium Heparin (REF: 367526, BD-Plymouth, UK), prepared as described in Kirwan et al. [28] and then stored at −20 °C pending analysis for plasma urea N, total protein, and creatinine concentrations.

In sacco DM degradability determinations were conducted on d 8 and d 9 in each experimental period to determine the extent of rumen digestion of each of the three carbohydrate sources offered (rolled barley, maize meal, and soya hulls) over a 48 h period.

In situ filter bags (5 × 10 cm; 50 µm pore size) (Ankom Technology, Macedon, New York, USA) containing approximately 5 g DM feed were placed inside large mesh nylon bags and inserted into the ventral sac of the rumen and secured with a metal weight. The in situ bags were inserted at 1700 h on d 8 of each experimental period and incubated for 0, 2, 4, 6, 8, 12, 24, 48 h in reverse order. All feed samples were previously ground using a Norris hammer mill fitted with a 2 mm screen (Lab Mill Christy Turner, Suffolk, UK). After removal from the rumen, all bags were immediately submerged in ice cold water, thoroughly washed and frozen at −20 °C. Upon thawing, in situ bags were rinsed in a domestic washing machine for 30 min using the cold rinse cycle in the absence of detergent, then dried at 55 °C for 48 h. Degradability constants *a*, *b*, and *c* were estimated according to the non-linear model: $p = a + b\,(1 - e^{-ct})$ [30], where '*a*' represents the soluble degradable fraction, '*b*' represents the slowly degraded fraction within the rumen and '*c*' is the constant rate of degradation per hour of the '*b*' fraction with time '*t*'. Effective degradability (ED) was calculated using the equation $a + [bc/(c + k)]$ [30], where k is the fixed rumen outflow rate 0.03 h^{-1} [31].

Rumen fluid samples were collected at 1, 2, 4, 6, 8 h post-feeding on d 10 and 11 while in the metabolism house via the cannula for pH, NH_3 and volatile fatty acids (VFA) determination as described previously in Kirwan et al. [28] and analysed for NH_3 concentrations using the phenol hypochlorite method of Weatherburn [32].

2.3. Chemical Analysis

Samples of TMR, concentrates, GS and faeces were prepared and analysed for DM, NDF, acid detergent fibre (ADF), starch, ash, N, gross energy, ether extract, and N in urine as described in Kirwan et al. [28]. The apparent digestibility (%) of nutrients [DM, organic matter (OM), crude protein (CP), NDF and starch] was calculated according to the following equation [33] (intake and output of nutrients in kilograms):

Apparent nutrient digestibility = (1 − (faecal nutrient/total nutrient intake)) × 100.

Data were analysed as a replicated 3 × 3 Latin Square design using the PROC MIXED procedure of Statistical Analysis Software (SAS v9.4, Inst. Inc., Cary NC, USA) [34]. Normal distribution and homogeneity of variance were analysed using the UNIVARIATE procedure. Animal within period was the experimental unit. Model consisted of animal, period, and dietary treatment. Animal within period was a random effect. Ruminal data collected at different times after feeding were analysed using the PROC MIXED procedure for repeated measures. The model contained the same fixed effects as before, except that time after feeding and its interaction with the main effects were included. Effects were considered significant at $p < 0.05$, with a tendency towards significant $p < 0.10$. When significant differences were detected, difference among treatment means and treatment by time point interaction were tested using Tukey's multiple comparison test.

3. Results

The effect of carbohydrate source on nutrient intake and total tract apparent digestibility of nutrients is presented in Table 2. There was no difference among dietary treatments for dry matter, OM, or CP intake ($p > 0.05$). Animals offered SH had a higher NDF and lower starch intake compared to animals offered RB and MM ($p < 0.001$) whereas animals offered MM had a higher starch intake compared to animals offered RB ($p < 0.05$).

Total tract apparent digestibility of DM, OM, and CP did not differ ($p > 0.05$) between dietary treatments; however, total tract digestibility of CP tended to be higher for RB compared to those offered MM ($p < 0.10$). Neutral detergent fibre total tract digestibility was higher for animals offered SH ($p < 0.001$) with no difference between animals offered RB and MM ($p > 0.05$). Starch total tract digestibility was higher for animals offered RB compared to animals offered MM ($p < 0.05$).

Table 2. The effect of concentrate carbohydrate source on nutrient intake and total tract apparent digestibility in beef heifers fed grass silage-based diets.

	Dietary Treatment [1]				
	RB	**MM**	**SH**	**SEM**	***p*-Value**
Intake (kg d $^{-1}$)					
Dry matter	6.04	6.03	6.03	0.031	0.958
Organic matter	5.65	5.62	5.60	0.028	0.442
Crude protein	0.89	0.87	0.87	0.011	0.577
Neutral detergent fibre	1.99 ^{b}	1.89 ^{c}	3.13 ^{a}	0.009	0.001
Starch	1.12 ^{b}	1.25 ^{a}	0.04 ^{c}	0.006	0.001
Apparent total tract digestibility, %					
Dry matter	76.24	74.90	75.03	0.635	0.311
Organic matter	77.74	76.33	76.62	0.643	0.310
Crude protein	72.02	66.82	68.12	1.396	0.061
Neutral detergent fibre	61.44 ^{b}	59.89 ^{b}	73.81 ^{a}	0.831	0.001
Starch [2]	96.89 ^{a}	95.67 ^{b}	-	0.328	0.039

^{abc} Within a row, means with a different superscript differ ($p < 0.05$). [1] RB rolled barley; MM maize meal; SH soya hulls. [2] Total tract apparent starch digestibility RB vs. MM.

The in sacco ruminal digestion kinetics and effective degradability of carbohydrates are presented in Table 3. Fraction *a* (rapidly degradable component) was different for all three carbohydrate sources ($p < 0.001$), 10% higher for rolled barley compared to maize meal, and 73% lower for soya hulls compared to rolled barley. The slowly degradable component *b* was 77% and 65% higher for soya hulls compared to rolled barley and maize meal, respectively ($p < 0.001$), while there was no difference between rolled barley and maize meal ($p > 0.05$). The factional rate of degradation per h of fraction *c* was higher for rolled barley ($p < 0.01$) compared to maize meal and soya hulls. Effective degradability was lower for soya hulls compared to rolled barley and maize meal ($p < 0.001$) which did not differ ($p > 0.05$).

Table 3. In sacco ruminal digestion kinetics [1] and effective degradability (ED) of carbohydrate sources fed to beef heifers on a grass silage-based diet.

DM [2]	**Rolled Barley**	**Maize Meal**	**Soya Hulls**	**SEM**	***p*-Value**
a	0.641 ^{a}	0.572 ^{b}	0.170 ^{c}	0.0045	<0.0001
b	0.246 ^{a}	0.381 ^{a}	1.106 ^{b}	0.0142	<0.0001
c	0.371 ^{a}	0.100 ^{c}	0.014 ^{c}	0.0383	0.001
ED	0.877 ^{a}	0.847 ^{a}	0.568 ^{b}	0.0181	<0.0001

^{abc} Within a row, means with a different superscript letter differ ($p < 0.05$). [1] Kinetics of digestions were estimated using the equation: $p = a + b\,(1 - e^{-ct})$, where a = soluble fraction, b = slowly degradable fraction, and c = fractional rate of degradation per hour of the '*b*' fraction with time '*t*'. *ED* calculated using the equation $a + [bc/(c + k)]$, $k = 0.03\ h^{-1}$. [2] Dry matter disappearance.

The effect of carbohydrate type on N balance and blood metabolites is presented in Table 4. Nitrogen intake (g d^{-1}) was not affected by dietary treatment ($p > 0.05$). In addition, dietary treatment had no effect on total N excretion (g d^{-1}), the amount of N retained (g d^{-1}), and the amount of N excreted in the urine (g d^{-1}) ($p > 0.05$). There was a higher percentage of N excreted in the faeces and a lower percentage of N excreted in the urine when animals were offered MM compared to those offered RB ($p < 0.05$).

Blood plasma urea concentrations were higher for animals offered RB ($p < 0.01$), while no differences were observed for plasma creatinine and blood glucose levels between treatments ($p > 0.05$).

Table 4. The effect of concentrate carbohydrate source on nitrogen balance and blood metabolites in beef heifers fed grass silage-based diets.

	Dietary Treatment [1]				
	RB	**MM**	**SH**	**SEM**	***p*-Value**
N intake (g d $^{-1}$)	142	143	143	4.0	0.105
N output (g d $^{-1}$)					
Urine N	81	76	82	4.0	0.553
Faecal N	39 [b]	46 [a]	43 [ab]	1.3	0.025
Total excretion	120	118	126	4.8	0.514
Retained	21.0	23.9	15.9	4.9	0.538
N recovery [2]					
Urine	0.57	0.51	0.57	0.031	0.250
Faeces	0.28	0.32	0.31	0.012	0.062
N excreted (%) [3]	85.19	83.21	85.21	2.360	0.777
NUE (%) [4]	14.81	16.79	14.79	2.360	0.777
% total excreted [5]					
Urine	67.20 [a]	61.70 [b]	64.74 [ab]	1.553	0.045
Faeces	32.80 [b]	39.30 [a]	35.26 [ab]	1.553	0.045
Urine metabolites					
Creatinine (µmol L^{-1})	183.4	215.2	180.4	55.99	0.882
Urea (mmol L^{-1})	5.72	6.13	8.46	1.30	0.352
Blood metabolites					
Urea (mmol L^{-1})	3.05 [a]	2.52 [b]	2.86 [b]	0.075	0.002
Creatinine (µmol L^{-1})	140.9	141.7	137.3	5.13	0.285
Glucose (mmol L^{-1})	3.76	3.77	3.83	0.050	0.616

[ab] Within a row, means with a different superscript letter differ (p <0.05). [1] Grass silage-based diets supplemented with either RB rolled barley, MM maize meal, or SH soya hulls. [2] N recovery = N out [faeces, urine (g/d)]/N intake (g/d); [3] N excreted = [faeces + urine output (g/d)]/N intake (g/d)*100; [4] NUE nitrogen use efficiency; [5] % total excreted = [urine, faeces output (g/d)/total N output (g/d)*100.

Table 5 shows the effect of carbohydrate source on rumen fermentation parameters. Animals offered SH had a higher ruminal pH than animals offered RB and MM (p < 0.001). Postprandial evolution of ruminal pH did not differ with dietary treatment (p > 0.05; Figure 1). Independent of dietary treatment, ruminal pH decreased, reaching nadir 1 h post-feeding, then gradually increasing to 6 h post-feeding (p < 0.001).

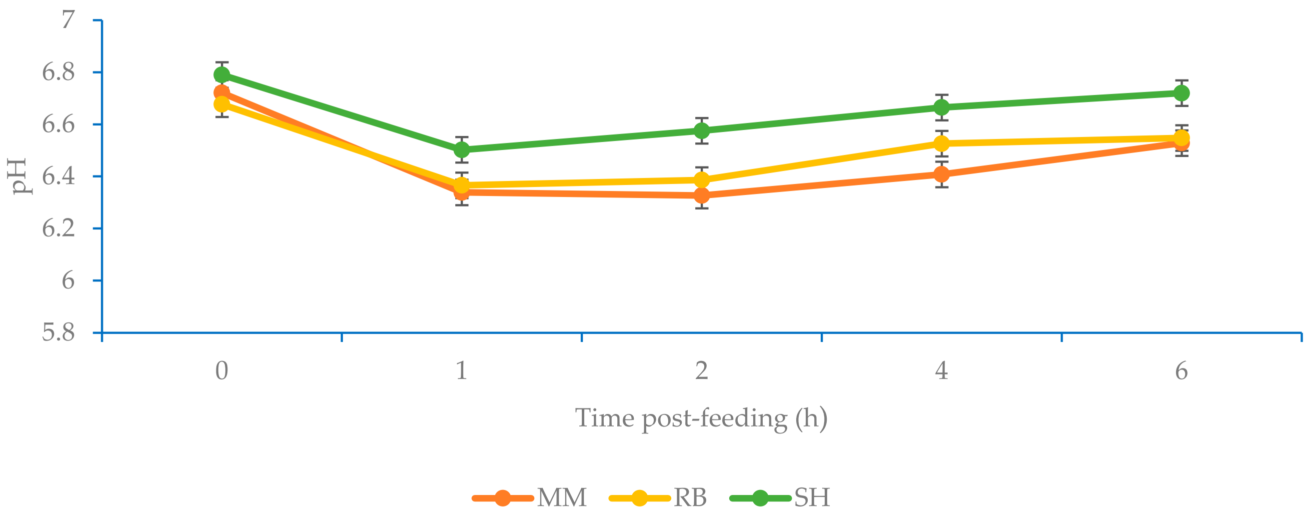

Figure 1. The effect of concentrate carbohydrate source on rumen pH in beef heifers fed grass silage-based diets supplemented with either RB rolled barley, MM maize meal, or SH soya hulls.

Table 5. The effect of concentrate carbohydrate source on rumen fermentation parameters in beef heifers fed grass silage-based diets.

	Dietary Treatment [1]					Time after Feeding							
	RB	**MM**	**SH**	**SEM**	**Diet**	**0 h**	**1 h**	**2 h**	**4 h**	**6 h**	**SEM**	**Time**	**Diet × Time**
pH (mmol L $^{-1}$)	6.50 ^{b}	6.46 ^{b}	6.65 ^{a}	0.040	<0.01	6.73 ^{a}	6.40 ^{d}	6.43 ^{d}	6.53 ^{bc}	6.60 ^{b}	0.037	<0.001	0.110
NH_3	2.80	2.70	2.61	0.133	0.53	1.95 ^{d}	2.98 ^{c}	4.03 ^{ab}	3.00 ^{bc}	1.56 ^{e}	0.125	<0.001	0.019
Acetic	66.53	66.33 ⸸	68.97 ⸸	0.801	0.080	66.82	66.86	68.43	66.94	67.36	0.982	0.747	0.153
Propionic	10.51 ⸸	9.94	9.68 ⸸	0.255	0.079	8.13 ^{d}	11.36 ^{b}	11.88 ^{ab}	9.86 ^{c}	8.98 ^{cd}	0.286	<0.001	0.772
Butyric	8.87 ⸸	8.27	7.59 ⸸	0.435	0.090	7.11 ^{d}	8.45 ^{c}	9.31 ^{a}	8.47 ^{bc}	7.89 ^{cd}	0.385	<0.001	0.584
Valeric	0.96 ^{a}	0.88 ^{b}	0.86 ^{b}	0.018	0.006	0.70 ^{e}	0.88 ^{cd}	1.11 ^{a}	0.98 ^{b}	0.84 ^{d}	0.024	<0.001	0.304
Isovaleric	1.40	1.36	1.44	0.067	0.733	1.29 ^{d}	1.38 ^{bcd}	1.63 ^{a}	1.42 ^{b}	1.29 ^{d}	0.047	<0.001	0.372
Isobutyric	1.36	1.36	1.37	0.050	0.983	1.37 ^{c}	1.21 ^{d}	1.54 ^{ab}	1.41 ^{bc}	1.30 ^{cd}	0.046	<0.001	0.447
Ac: Pr [2]	6.53 ^{b}	7.07 ^{a}	7.49 ^{a}	0.145	0.003	8.40 ^{a}	6.13 ^{e}	5.90 ^{de}	6.99 ^{c}	7.74 ^{b}	0.173	<0.001	0.507
TVFA [3]	89.66	88.15	90.05	1.319	0.508	85.48 ^{ac}	90.18 ^{ab}	93.93 ^{ab}	89.15 ^{ac}	87.70 ^{c}	0.173	<0.001	0.345

$^{a–e}$ Within a row, means with a different superscript letter differ ($p < 0.05$). ⸸ Tendency towards significant ($p < 0.10$). [1] Grass silage-based diets supplemented with either RB rolled barley, MM maize meal, or SH soya hulls. [2] Ac: Pr = ratio of acetic acid to propionic acid (acetic:propionic). [3] TVFA = total volatile fatty acids.

There was a treatment × time interaction for rumen NH_3 concentrations ($p < 0.01$) (Figure 2). At 1 h post-feeding, animals offered the MM had higher rumen NH_3 concentrations than those offered the RB ($p < 0.05$), but this response was reversed at 4 and 6 h post-feeding ($p < 0.01$), while at 6 h post-feeding, NH_3 concentrations for the animals offered RB were higher than those offered the SH ($p < 0.05$). There were no differences observed between dietary treatments for ruminal NH_3 concentrations ($p > 0.05$). The animals offered RB had a higher concentration of ruminal valeric acid than those offered SH and MM ($p < 0.05$). No differences were observed between dietary treatments for ruminal; acetic acid, propionic acid, butyric acid, branched chain fatty acids (isovaleric acid and isobutyric acid) and total rumen VFA concentrations ($p > 0.05$). However, ruminal acetic acid concentrations tended to be higher for animals offered SH compared to MM ($p < 0.10$), and animals offered RB tended to have higher ruminal propionic acid and butyric acid concentrations compared to animals offered SH ($p < 0.10$). Animals offered RB had lower ($p < 0.05$) Ac:Pr compared to animals offered SH and MM, which did not differ ($p > 0.05$) among each other. Concentrations of ruminal butyric acid, valeric acid, and isovaleric acid concentrations were highest 2 h after feeding ($p < 0.001$).

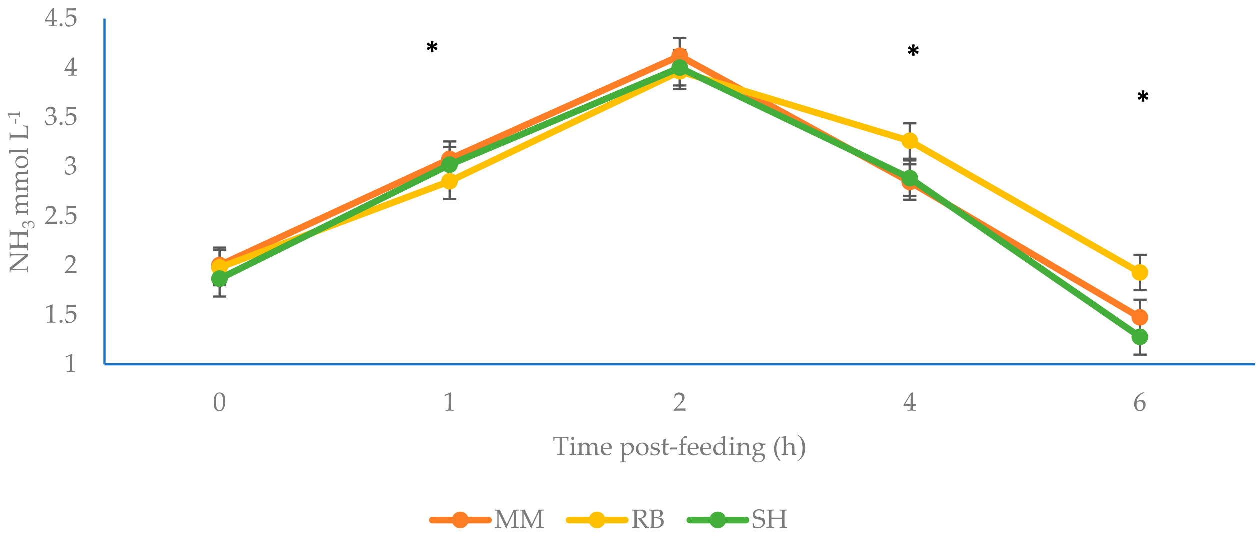

Figure 2. The effect of concentrate carbohydrate source on rumen ammonia concentrations in beef heifers fed grass silage-based diets supplemented with either RB rolled barley, MM maize meal, or SH soya hulls. * Denotes treatment × time interaction.

4. Discussion

The hypothesis that offering a carbohydrate source that is rapidly degraded within the rumen would capture more N within the rumen and in turn reduce N excretion was rejected.

4.1. In Sacco Degradability

The results obtained in this study from the in sacco degradability of the three feed ingredients fed reveal the difference in ruminal DM degradation of each carbohydrate source. In cereal grains, starch generally represents a large proportion of the feed DM, with a positive correlation between the ED of DM and ED of starch [35]. The high values obtained for fraction '*a*' (the rapidly degradable component) with rolled barley and maize meal indicate that most of the starch was immediately washed out upon immersion of the bags within the rumen. However, the high solubility rate obtained with these ingredients may have been over estimated due to mechanical particle loss [36] or the smaller particle size of barley and maize compared to that of soya hulls [37–39]. The animals used herein were fed at maintenance and to account for the underestimation in the digestibility of nutrients due to higher rumen turnover rates, the rumen outflow rate was fixed at 0.03 h^{-1} [40].

4.2. N-Balance

In the current study, N recovered in the urine was similar across all treatments at 55% of ingested N, whereas N recovered in the faeces tended to be higher for animals offered MM compared to those offered RB (32% vs. 28%, respectively). The partitioning of N excreted into urine and faeces is largely dependent on diet, with up to 75% of N excreted in urine when high protein, high concentrates diets are fed [41,42]; but can be reduced to 52% excreted N in urine when diets are formulated to NRC recommended CP concentrations [43]. Similarly, Colmenero and Broderick, [44] observed that dairy cows fed increasing levels of CP and RDP had higher ruminal NH_3 concentrations, resulting in higher levels of N excreted in the urine.

Urinary N excretion is an environmental concern as it is a major contributor to NH_3 emissions because urea in the urine is rapidly hydrolysed to NH_3 due to the prevalence of urease in the faeces [45]. Ammonia is the principle source of urea that is produced in the rumen from RDP fed to excess or an insufficient energy supply to rumen microbes, metabolised to urea in the liver and excreted in the urine [44]. It was hypothesised in this study that offering rolled barley, which has a more rapid rate of ruminal fermentation than maize meal and soya hulls would capture more NH_3 within the rumen and lead to a reduction in urinary N excretion. However, urine excretion was unaffected by carbohydrate source and was the major route of N excretion across all dietary treatments (79 g d^{-1}). Ferreira et al. [46] observed that replacing maize corn with increasing levels of SH in the diets of lambs increased urinary excretion. This increase in urinary excretion can be explained by the increase in DMI intake as the level of SH in the diet increased, while simultaneously increasing the intake of CP in the diet. Similarly, Yan et al. [47] established that the correlation between N intake and DMI is positive. Therefore, in this study, feed intake was restricted to maintenance, to ensure that DMI had no influence on N intake due to difference in energy density between the three feed ingredients [46], in addition to diets formulated to be isonitrogenous (142.6 g d^{-1}). The intake level of carbohydrates in the diet can impact the level of N excreted in the urine, as the rate and extent of carbohydrate fermentation within the rumen determines the utilisation of ruminal NH_3 for microbial synthesis [48] and the type of protein therein [49].

Offering maize meal, which is more resistant to rumen degradation compared to rolled barley [35], increases the percentage of total N excreted in the faeces by 39.30 vs. 32.80 %, respectively. Surber and Bowman [50] reported similar findings with beef cattle offered maize meal, where degradation of maize starch within the rumen was lower than those offered rolled barley leading to higher levels of N excreted in the faeces (35 vs. 30 g d^{-1}). The site and the extent of carbohydrate fermentation can influence the level of faecal N excretion. Faecal N is primarily of microbial origin with lesser amounts of undegraded feed protein and endogenous secretions [51]. Despite no differences in urinary N excretion observed between treatments in this study (79 g d^{-1}), the animals offered MM excreted a higher amount of N in the faeces compared to animals offered RB. Despite the higher level of starch intake with the animals offered MM, the animals offered RB had a higher apparent total tract digestibility of starch in addition to a tendency for a higher apparent total tract digestibility of CP, which would suggest undigested protein in the starch/protein matrix with animals offered MM [52]. Maize starch is more resistant to rumen degradation than other cereal grains, as the starch granules in maize are embedded in the protein matrix, prolamins, which are more resistant to degradation at higher pH [53].

4.3. Rumen pH

Rumen pH is a critical factor in the normal and stable function of the rumen because of its profound effect on microbial populations and fermentation products, and on physiological functions of the rumen, with typical ruminal pH in grain fed beef cattle ranging from 5.5 to 6.2 [54]. As the forage to concentrate ratio of the diet is decreased with high dietary levels of rapidly fermentable carbohydrates such as starch with low levels of effective fibre the probability of acidosis increases. Low ruminal pH may have been anticipated in the current study with the high concentrate to forage ratio offered. However, as a result of animals being fed to maintenance, and for additional rumen fill, all diets were supplemented with 1 kg DM of barley straw. The provision of straw in the diet enhances the level of fibre and physically effective fibre in the rumen, promoting rumination and saliva secretion, helping to buffer the acids from the fermentation of the feed. Additionally, higher pH values obtained in this current study may be associated with the decreased volume of rumen digesta (low DMI) and increased dilution rate of rumen liquid or because of the increased extent of chewing [55].

4.4. Rumen NH_3 Concentration

There was a time by treatment interaction in ruminal NH_3 concentrations, where the initial increase in ruminal NH_3 concentration with the animals offered MM is likely as a response to the lower availability of carbohydrate in the MM compared to the other dietary treatments [56]. Additionally, the lower levels of NH_3 associated with RB and SH in the initial 3 h post-feeding suggest that more energy was available to allow for better capture of NH_3 by the rumen microbes [57]. Across all dietary treatments, the highest ruminal NH_3 concentration was detected 2 h after feeding as a response to the rapid degradation of all sources of dietary protein similar to findings of Grigsby et al. [58]. There was no difference in mean ruminal NH_3 concentration between treatments. However, the overall mean ruminal NH_3 concentration was 2.48 mmol L^{-1}, which was lower than those reported in [59] but similar to [60]. Kang-Meznarich and Broderick, [61] reported 1.94 to 5 mmol L^{-1} as the optimum level of ruminal NH_3 concentration adequate for microbial synthesis and fibre digestion, suggesting the levels of ruminal NH_3 produced in this study were adequate.

4.5. VFA Concentrations

The concentrations of VFA within the rumen are the net result of substrate consumed by the animal and their absorption rate [62], with the rate of absorption increasing as the ruminal pH decreases [63]. The total average rumen VFA concentrations observed in this study (90.43 mmol L^{-1}) were lower compared to similar studies (148 mmol L^{-1}) involving beef cattle offered carbohydrates differing in rumen degradation rates [60]. However, these diets were offered ad libitum, whereas, in this current study DMI was restricted to maintenance. As the mean ruminal pH in this current study never dropped below 6.0, the lower rumen VFA concentrations were more likely as a result of lower rumen VFA production due to lower DMI consumed [55] rather than greater VFA absorption through the rumen epithelium [64]. While not significant, the higher concentrations of acetic acid observed with the animals offered SH is a result of the higher proportion of NDF within the diet and higher total tract digestibility of NDF in animals offered SH [55] and as a consequence resulted in a higher acetic acid: propionic acid ratio compared to the animals offered RB. The starch contained in barley is more fermentable within the rumen compared with maize starch [35]. However, the similar VFA concentrations observed in this study may be as a result of the different levels of processing associated with each ingredient [65] compared to the dry rolling of barley, maize grain was finely ground which produced large numbers of fine particles, increasing the surface area of the endosperm for utilisation by the rumen micro-organisms [66]. Similar observations were noted when substituting maize meal with rolled barley in beef cattle [67] and with dairy cows [68,69] and substituting maize meal with soya hulls [70].

5. Conclusions

Offering a carbohydrate source that is rapidly degraded within the rumen such as rolled barley did not alter ruminal NH_3 concentrations, and thus reduce N excretion in beef heifers offered GS-based diets fed to maintenance. Similar ruminal NH_3 concentrations were observed across all treatments, highlighting that protein degradation exceeded carbohydrate fermentation 2 h post-feeding. In conclusion, supplementing grass silage-based diets with concentrate carbohydrate sources with different fermentation profiles had no effect on N metabolism of beef heifers fed to maintenance. However, this approach is unlikely in practice as the animals were fed to maintenance on a diet that contained 60% concentrates.

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