Effect of forage source and a supplementary methionine hydroxy analog on nitrogen balance in lactating dairy cows offered a low crude protein diet¹

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ABSTRACT

Four primiparous and 4 multiparous midlactation dairy cows were stratified by pre-experimental milk yield (23.5 ± 2.3 kg/d), protein yield (0.75 ± 0.066 kg/d), parity, and days in lactation (121 ± 10 d) into 4 groups of 2 in a 2×2 factorial, Latin square design ($n = 8$) to assess the effect of forage source and a supplementary methionine hydroxy analog on nitrogen (N) balance where low crude protein (CP) diets (13.3%) are offered. Diets contained either predominantly grass silage [GS (G– and G+)] or corn silage [CS (C– and C+)] as the forage source and were offered with (G+ and C+) or without (G– and C–) the isopropyl ester of 2-hydroxy-4 methylthio butanoic acid (HMBi). The G– and G+ contained 46% GS and 10% CS in the dry matter (DM), whereas C– and C+ contained 12% GS and 52% CS in the DM. Supplementary HMBi was included at a rate of 0.2% of DM in G+ and C+ diets. Diets were isonitrogenous ($9.8 \pm 0.4\%$ protein truly digested in the small intestine) and isoenergetic (0.96 ± 0.01 units of energy for lactation; kg/DM). Each of the 4 experimental periods lasted 24 d: 14 d for dietary adaptation, followed by 10 d of housing in individual metabolism stalls; N balance was conducted on the last 5 d of each experimental period. Intake of DM was higher for CS-based vs. GS-based diets (20.23 vs. 18.41 kg/d). No effect of dietary treatment was found on milk yield or yields of milk fat, protein, and lactose. Supplementing with HMBi tended to improve milk solids yield (1.69 vs. 1.59 kg/d), casein yield (0.59 vs. 0.55 kg/d), and concentrations of casein (2.89 vs. 2.73%) and protein (3.58 vs. 3.49%) in the milk. Dietary N intake was higher for CS-based vs. GS-based diets (0.460 vs. 0.422 kg/d). However, forage source or supplementary HMBi had no effect on N excretion in the feces, urine, or milk. Excretion of urinary urea was

positively related to N intake. Concentrations of urea N in the plasma (2.34 vs. 1.72 mmol/L), milk (2.54 vs. 2.24 mmol/L), and urine (123.32 vs. 88.79 mmol/L), and total excretion of urinary urea N (40.23 vs. 35.09 g/d) were higher for animals offered CS-based vs. GS-based diets. Corn silage improved N intake through improved DM intake. However, neither forage source nor HMBi supplementation affects N output in the feces, urine, or milk.

Key words: dairy cow, nitrogen balance, forage, methionine

INTRODUCTION

The quantity of N excreted by the dairy cow is of environmental concern. Excreted N, and in particular the urinary N components urea, creatinine and allantoin, have the potential to contribute to environmentally detrimental gases such as NH_3 and N_2O (Pakro and Dillon, 1995). Irish agriculture accounts for 98 and 79% of national NH_3 and N_2O emissions, respectively (Hyde et al., 2005; O Bric, 2005). International obligations under the Kyoto Protocol and the Gothenburg Agreement have set targets for the reduction in both of these gases. In Ireland a 9% decrease in NH_3 emissions from 1990 levels is required (Hyde et al., 2003), whereas reductions in greenhouse gasses (of which N_2O is a contributor) have been set at 20% below 2005 levels (European Commission, 2008). Because the storage and land application of dairy cow excreta accounts for a significant portion of N losses to the environment (Hyde et al., 2003), it is of interest to develop strategies that will decrease N excretion from the dairy cow and to this end, dietary manipulations offer great potential.

Decreasing the level of dietary CP has been shown to have a beneficial effect on N excretion by dairy cows (Leonardi et al., 2003; Olmos Colmenero and Broderick, 2006), and particularly urinary N excretion (Mulligan et al., 2004). It has been shown that where dietary CP concentration is decreased (from 18.4 to 15.1% CP), there is little effect on milk yield or milk protein yield (Broderick, 2003; Olmos Colmenero and Broderick, 2006). However, others have suggested that in situations where low dietary CP are offered, AA

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may become limiting. Methionine is said to be the first limiting AA for milk production (NRC, 2001) and as such has received a great deal of attention as an AA which can improve milk N output at a given level of CP (Phipps et al., 2010) or, where dietary CP is decreased, maintain milk N output at levels comparable to higher-CP diets (Broderick et al., 2008). Additionally, strategies that improve the balance of AA available to the animal can decrease the potential for post-ruminal sources of N for excretion (Robinson, 2010).

Further manipulation of the diet is possible through altering the forage source offered to the dairy cow. Grass silage (**GS**; predominantly *Lolium* spp.) and corn silage (**CS**; *Zea mays*) are the principal forages used in Northern Europe (Rath and Peel, 2005). Grass silage is noted for its highly rumen-degradable N and low levels of fermentable OM (**FOM**). This asynergy between available energy and available N can result in poor capture of dietary N by the rumen microbes (McDonald et al., 2002) and excessive concentrations of $\text{NH}_3\text{-N}$ in the rumen. Consequently, high levels of N are excreted, principally in the urine. In recent years, the use of CS in dairy diets has become more prominent in Ireland, thanks mainly to varietal improvements (O'Mara et al., 1998). Corn silage contains a higher concentration of FOM relative to GS, thus providing an energy source for the capture of dietary N. Replacing a portion of GS with CS in the diets of dairy cows has been shown to improve DMI, milk yield, and efficiency of N use (Mulligan et al., 2002; Burke et al., 2007). However, CS is low in CP; therefore, protein supplementation is often required to maintain dietary CP (O'Mara et al., 1998; Burke et al., 2007).

The objective of the current experiment was to evaluate the effect of forage source (GS vs. CS) on N balance in lactating dairy cows consuming low-CP diets offered with or without supplementary methionine.

MATERIALS AND METHODS

Animals and Management

All procedures described in this experiment were conducted under experimental license from the Irish Department of Health and Children in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendment of the Cruelty to Animals Act 1876) Regulations, 1994 (<http://www.irishstatutebook.ie/1994/en/si/0017.html>).

Four primiparous and 4 multiparous dairy cows (*Bos taurus*, strain Holstein-Friesian) were selected from the autumn calving dairy herd at University College Dublin (UCD) Lyons Research Farm (Newcastle, Dublin, Ireland; 53°17'56"N, 6°32'18"W). Animals were selected

based on pre-experimental milk and milk protein yield, parity, and days in lactation, and assigned to 1 of 4 dietary treatments in a 2×2 factorial, Latin square design ($n = 8$). The multiparous cows were fitted with rumen cannulas (Bar Diamond Inc., Parma, ID) to facilitate the sampling of rumen contents. Animals were 121 ± 10 d in lactation before measurements commenced. The multiparous cows had previous 305-d lactation yields of $7,133 \pm 454$ kg for milk and 240 ± 4 kg for protein, whereas the average pre-experimental milk yield was 23.5 ± 2.3 kg/d with a fat yield of 0.87 ± 0.065 kg/d and a protein yield of 0.75 ± 0.066 kg/d.

Each experimental period consisted of a 14-d dietary adjustment period whereby cows were allocated their respective diets using individual pre-programmed feed boxes (RIC System; Insentec B.V., Marknesse, the Netherlands), followed by a 10-d period where animals were housed in individual metabolism stalls to facilitate sample collection. Sample collection for N balance commenced on d 5 of each 10-d sampling period and ran for 5 d.

Diets and Experimental Design

The GS used during this experiment consisted of predominantly perennial ryegrass (*Lolium perenne*). The crop was felled using a mower-conditioner during the early booth stage of vegetation (growth stage 41; Zadoks et al., 1974), wilted for 16 h, and harvested with a precision chop forage harvester (mean particle length 50 mm). The crop was then ensiled under a black polythene cover without the use of an additive. Corn silage (*Z. mays*, variety Justina) was grown with the aid of plastic mulch. The crop was harvested at the dough stage (growth stage 85; Lancashire et al., 1991) using a precision chop forage harvester (mean particle length 25 mm). Ensilage was without the use of an additive under a black polythene cover. Prior to the commencement of the experiment, samples of GS and CS were taken from the respective silos. Fresh samples were sent to a forage laboratory [Agri-Food and Biosciences Institute (AFBI) Hillsborough, Co. Down, Northern Ireland] for near infrared (NIR) analysis (Foss NIR-Systems 5000; Foss UK Ltd., Warrington, Cheshire, United Kingdom). Additional samples were frozen at -20°C and sent on dry ice (solidified carbon dioxide; Polar Ice Ltd., Portarlinton, Co. Laois, Ireland) for analysis of AA composition (Laboratory Carte; Adisseo France S.A.S., Commentry, France). The chemical composition of the forages used is presented in Table 1.

The experiment was a 2×2 factorial design, 2 forage sources (GS vs. CS) offered with (0.2% of DM) or without (0% of DM) supplementary methionine. A commercially available source of methionine was used

Table 1. Chemical composition of forages used in experiment

Item ¹	Forage	
	Grass silage	Corn silage
DM (%)	23.2	28.7
Composition (%)		
CP	10.8	8.9
NDF	52.0	48.5
ADF	24.0	27.3
Starch	—	25.8
DMD	67.5	69.5
UFL	0.78	0.85
PDIN	6.3	5.5
PDIE	6.3	6.9
Lysine (% of dAA)	5.4	4.5
Methionine (% of dAA)	1.9	2.0

¹DMD = digestibility of DM; UFL = unit of energy for lactation; PDIN = protein digested in the small intestine, where nitrogen is limiting microbial protein synthesis; PDIE = protein digested in the small intestine, where energy is limiting microbial protein synthesis; dAA = digestible AA.

in this experiment (MetaSmart Dry; Adisseo France S.A.S.). The source of methionine contained a minimum value of 95% isopropyl ester of 2-hydroxy-4-methylthio butanoic acid (**HMBi**), supplied as a 60% premix with silica as an inert carrier. The dietary treatments were as follows; GS without supplementary HMBi (**G−**), GS with supplementary HMBi (**G+**), CS without supplementary HMBi (**C−**), and CS with supplementary HMBi (**C+**).

Table 2 presents the composition of each dietary treatment. Diets were formulated such that the forage DM component consisted of 82% GS and 18% CS (**G−** and **G+**) or 82% CS and 18% GS (**C−** and **C+**). Methionine (**HMBi**) was supplemented in both **G+** and **C+** diets at the rate of 0.2% of DM; barley straw was supplemented in **C−** and **C+** at the rate of 2% of DM as a source of effective fiber. The concentrate component consisted of rolled barley, sugar beet pulp, soybean meal, and a mineral/vitamin premix. The forage-to-concentrate ratio was 56:44 and 64:36 for the GS-based and the CS-based diets, respectively.

All diets were formulated using Ruminix software (A-Systems, Versailles, France) to meet the energy [18.71 U of energy for lactation (**UFL**)/d], protein [protein truly digested in the small intestine (**PDI**); 1,950 g/d], and lysine (7.1% of **PDI**) requirements for production of 30 kg of milk/d containing 4.1% fat and 3.3% protein for a 600-kg animal. Diets that were not supplemented with HMBi (**G−** and **C−**) were calculated to contain methionine at 1.8% of **PDI**, whereas **G+** and **M+** were calculated to contain methionine at 2.3% of **PDI** (Table 2). Diets were offered once daily as a TMR, with allowances made for refusals amounting to 10% of DM of

fered. Mixing was achieved using an experimental diet feeder (American Calan Inc., Northwood, NH).

Nitrogen Balance Study

Following acclimation to the metabolism house, the N balance study ran for 5 consecutive days in each experimental period. To facilitate the separate collection of urine and feces, animals were fitted with a specially constructed harness system. This allowed the urine to flow through a pipe into a plastic container that contained 150 mL of 25% H₂SO₄ to prevent N loss as NH₃.

Feces were collected in trays behind each animal. Urine, feces, and feed refusals were weighed daily following the a.m. milking. Feed was weighed and offered once daily after all collections and sampling were complete. Milk output was recorded at a.m. and p.m. milking using the Weighall milk meter system (Dairy-master, Causeway, Co. Kerry, Ireland).

Sample Collection

Samples of GS, CS, feed refusals, and each TMR were taken daily and pooled for each N balance study. Subsamples were then dried at 55°C for 48 h for routine chemical analysis with additional subsamples frozen at −20°C for analysis of total N. Feces were mixed and a sample was collected each morning. These samples were then split: 1 subsample was dried at 55°C for 240 h, with a further subsample frozen to −20°C pending chemical analysis. Subsamples of dried and fresh feces were composited on an animal basis for each N balance study. Urine samples were taken each morning and immediately frozen to −20°C. Milk samples were taken at a.m. and p.m. milkings, pooled in proportion to yield, and then frozen to −20°C pending analysis of milk N. Milk samples were taken during a.m. and p.m. milking and pooled according to production on a per cow basis for each N balance experimental period.

On d 1 and 5 of each N balance study, blood samples were taken by jugular venipuncture into Vacutainers containing K₂EDTA (no. 367525; Becton, Dickinson and Co., Plymouth, UK) and centrifuged at 2,100 × *g* for 20 min at 4°C for extraction of plasma. These samples were then frozen at −20°C pending analysis for plasma urea N (**PUN**).

Rumen fluid samples were harvested from cannulated cows every 2 h over a 48-h period for determination of rumen NH₃-N levels. Rumen fluid was obtained using a collection tube (#RT; Bar Diamond Inc.) and a 60-mL disposable syringe. Four-milliliter subsamples were drawn off using an automatic pipette and acidified by mixing with 1 mL of trichloroacetic acid (50% wt/vol) before freezing at −20°C.

Table 2. Ingredient composition and chemical composition of dietary treatments

Item	Dietary treatment ¹			
	G–	G+	C–	C+
Ingredient composition (% of DM)				
Grass silage	46.0	46.0	12.0	12.0
Corn silage	10.0	10.0	52.0	52.0
Barley straw	0	0	2.0	2.0
Rolled barley	21.0	21.0	8.0	8.0
Beet pulp	13.0	13.0	12.0	12.0
Soybean	9.0	9.0	13.0	13.0
MetaSmart ²	0	0.2	0	0.2
Limestone ³	0	0	0.5	0.5
Vitamins/minerals ⁴	1.0	1.0	1.0	1.0
Grass:corn	82:18	82:18	18:82	18:82
Forage:concentrate	56:44	56:44	66:34	66:34
Chemical composition (% of DM)				
DM (g/kg)	36.3	36.5	40.0	41.0
DMD ⁵	74.4	72.5	73.0	72.2
Digestible N	56.1	56.4	57.9	57.1
CP	13.3	13.2	13.3	13.4
Starch	14.6	14.7	17.8	17.7
NDF	50.0	51.4	59.1	58.3
NDF _{forage} ⁶	28.8	28.8	31.5	31.5
ADF	21.6	20.5	22.3	22.9
Ash	6.4	6.2	6.0	5.8
UFL ⁷	0.91	0.90	0.92	0.90
PDIN ⁸	9.3	9.3	10.1	10.1
PDIE ⁹	10.5	10.5	10.1	10.1
PDIA ¹⁰	4.4	4.4	5.5	5.5
Lysine	0.54	0.54	0.54	0.53
Methionine	0.15	0.20	0.15	0.19
Lysine:methionine	3.65:1	2.74:1	3.53:1	2.79:1

¹G– = grass silage-based TMR without supplementary HMBi, G+ = grass silage-based TMR with supplementary HMBi, C– = corn silage-based TMR without supplementary HMBi, C+ = corn silage-based TMR with supplementary HMBi. HMBi = isopropyl ester of 2-hydroxy-4-methylthio butanoic acid.

²MetaSmart (Adisseo France S.A.S., Commeny, France) consists of not less than 57% monomer esters of HMBi, 4% maximum humidity.

³Limestone consists of ground rock limestone, Ca 39%.

⁴Vitamins/minerals consist of the following: retinol (300,000 IU/kg), cholecalciferol (60,000 IU/kg), α -tocopherol (1,500 mg/kg), calcium carbonate, monocalcium phosphate, calcium chloride, magnesium oxide, molasses, iodine (CaI_2O_6 , 520 mg/kg), copper (CuO_4S , 4,000 mg/kg), manganese ($\text{C}_{18}\text{H}_{14}\text{MnN}_2\text{O}_{2+2}$, 2,400 mg/kg), cobalt (CCoO_3 , 80 mg/kg), zinc (O_4SZn , 4,000 mg/kg), selenium ($\text{Na}_2\text{O}_3\text{Se}$, 40 mg/kg), Ca 6%, Na 8%, Mg 4%, P 6%.

⁵Digestibility of DM.

⁶NDF_{forage} = contribution of the forage component of the DM to NDF.

⁷Unit of energy for lactation.

⁸PDIN = protein truly digested in the small intestine, where nitrogen is limiting microbial protein synthesis.

⁹PDIE = protein truly digested in the small intestine, where energy is limiting microbial protein synthesis.

¹⁰PDIA = feed protein truly digested in the small intestine.

Sample Analysis

Dried feed, feed refusals, and feces were ground in a hammer mill fitted with a 1-mm screen (Christy Turner Ltd., Suffolk, United Kingdom). Neutral detergent fiber and ADF concentrations were determined by the method of Van Soest et al. (1991) using the Ankom 220 Fiber Analyzer (Ankom Technology Corp., Fairport, NY). Ash content was determined following combustion in a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at 550°C for 5 h. Starch content was determined using the Megazyme Total

Starch Assay Procedure (product no: K-TSTA; Megazyme International Ireland Ltd., Wicklow, Ireland). The N content of feed and feces was determined using the micro-Kjeldahl technique. Briefly, samples were weighed and placed into flasks for a block digestion unit (model no. 435; Büchi Labortechnik AG, Postfach, Switzerland) once digested samples were distilled (model no. 323; Büchi Labortechnik AG) into 50 mL of boric acid (2% wt/vol) containing Tashiro's indicator before titration. The N content of urine was determined by combustion (Leco FP-528 N Analyzer; Leco Corp., St Joseph, MI). Digestibility of DM (**DMD**)

was calculated as follows; $DMD = (DMI - \text{fecal DM output})/DMI$.

Feed and feces gross energy (**GE**) were determined using bomb calorimetry (Parr 1281 bomb calorimeter; Parr Instrument Co., Moline, IL). From these values, digestible energy (**DE**) and energy digestibility (**dE**) were calculated as follows: $DE = (DMI \times \text{feed GE}) - (\text{feces DM out} \times \text{GE of feces})$ and $dE = 1 - [(\text{feces DM out} \times \text{feces GE}) \div (DMI \times \text{feed GE})]$. Crude fiber (**CF**) was determined using AOAC (1990) methodology (method number 978.10) adopted for use in the Ankom 220 Fiber Analyzer (Ankom Technology Corp.). Feed N content was determined using the micro-Kjeldahl technique, as described previously, and CP was calculated as $CP = N \times 6.25$. These data were then applied to the following formula to calculate feed ME: $ME = \text{feed GE} \times dE \times ME:DE$, where $ME:DE = 0.8417 - (9.9 \times 10^{-5} \text{ CF}) - (1.196 \times 10^{-4} \text{ CP}) + (0.221 \times L) \pm 0.0087$, and L = feeding level (Jarrige, 1989). The efficiency of use of ME for lactation (**kl**) was calculated as follows: $kl = 0.463 + (0.24 \times ME \div GE)$. From these calculations, the UFL content of each TMR were calculated as follows: $UFL = ME \times kl \div 7.1128$ (Jarrige, 1989). Organic matter content of each TMR was calculated as $100 - \text{percentage ash}$ and FOM content as $OM - \text{fermentation products} - \text{ether extract}$ (Jarrige, 1989).

Values for feed protein truly digestible in the small intestine (**PDIA**); protein truly digestible in the small intestine, where N is limiting microbial protein synthesis (**PDIN**); and protein truly digestible in the small intestine, where energy is limiting microbial protein synthesis (**PDIE**) of each dietary treatment were calculated using the following equations of Jarrige (1989): $PDIA = 1.11 \times CP \times (1 - \text{deg}) \times dsi$, $PDIN = PDIA + [0.64 \times CP \times (\text{deg} - 0.1)]$, and $PDIE = PDIA + (FOM \times 0.093)$, where deg is the theoretical degradability of feeds in sacco and dsi is the true digestibility of undegraded dietary protein in the small intestine. Both deg and dsi were obtained from published data (Jarrige, 1989) whereas CP was determined as described previously for feed.

Milk samples were prepared for analysis as per the International Dairy Federation (IDF; method 29-2:2004; ISO, 2004). Milk N was analyzed by combustion (Leco FP-528 N Analyzer; Leco Corp.) and CN N was analyzed using the micro-Kjeldahl technique as per the IDF (method 29-2:2004; ISO, 2004). Urea levels in blood, milk, and urine were determined using the Randox Urease-Berthelot colorimetric method (Cat no. UR1068; Randox Laboratories, Crumlin, Antrim, Northern Ireland) as described previously by Mulligan et al. (2004). Samples of rumen fluid were allowed to thaw for 16 h to 4°C, centrifuged at $2,100 \times g$ for 10

min at 4°C. One milliliter of supernatant was drawn off and diluted 1 in 5 with distilled water (dH_2O), centrifuged at $1,600 \times g$ for 15 min at 4°C, 200 μL of supernatant were drawn off, and $\text{NH}_3\text{-N}$ concentrations were determined using the phenol-hypochlorite method of Weatherburn (1967). Microbial CP yield was determined through measurement of urinary allantoin using the method of Borchers (1977).

Statistical Analysis

Data from the N balance study were checked for adherence to the normal distribution and homogeneity of variance using histograms and formal statistical tests as part of the univariate procedure (SAS Institute, 2004). The relationship between urea N excretion (g/d) and feed N intake (kg/d) was tested for linear effects using Proc GLM (SAS Institute, 2004). Analysis of data was carried out using the repeated measures function in Proc Mixed (SAS Institute, 2004). The model included tests for the random effects of cow and the fixed effects of period, parity, and dietary treatment, and the interaction between dietary treatments. Where no interactions were observed for measures taken, this term was removed and only main effects are reported hereafter. Statistical significance was assumed at a value of $P < 0.05$, whereas a tendency toward significance was assumed at $P > 0.05$ but < 0.10 .

RESULTS

DMI, Milk Production, and Milk Composition

Table 3 shows the effect of forage source and supplementary HMBi on DMI, energy intake, milk yield (**MY**), milk constituent yield, and milk composition. Animals offered CS-based diets had a greater DMI ($P < 0.01$) and energy intake ($P < 0.01$) than those offered GS-based diets. Animals offered G+ had a lower energy intake than those offered C- ($P < 0.01$) and C+ ($P = 0.01$) but not G- ($P = 0.75$). Energy intake for animals offered C+ was not different from those offered G- or C- ($P > 0.05$). There was no effect ($P > 0.05$) of either forage source or HMBi supplementation on MY or yields of fat, protein, and lactose. Milk solids yield improved with HMBi supplementation ($P = 0.09$) versus nonsupplemented diets. Similarly, animals supplemented with HMBi had greater yields of CN ($P = 0.09$) and higher concentrations of CN ($P = 0.01$) and protein ($P = 0.09$) in the milk than those offered nonsupplemented diets. Milk protein concentration was higher from animals offered C+ than from animals offered C- ($P = 0.06$) and G- ($P = 0.04$). Milk protein concentration from animals offered G+ were not dif-

Table 3. The effect of forage source and supplementary isopropyl ester of 2-hydroxy-4-methylthio butanoic acid (HMBi) on DMI, energy intake, milk production, and milk composition

Item	Dietary treatment ¹				SEM	Significance		
	G–	G+	C–	C+		Forage	HMBi	Interaction
DMI (kg/d)	18.4 ^b	18.4 ^b	20.4 ^a	20.1 ^a	0.737	<0.01	0.85	0.79
UFL ² intake (per day)	16.8 ^{bc}	16.5 ^c	18.6 ^a	18.0 ^{ab}	0.796	<0.01	0.39	0.69
Output (kg/d)								
Milk	19.7	20.8	20.6	19.9	0.943	0.92	0.74	0.13
Milk solids	1.52	1.68	1.66	1.71	0.091	0.18	0.09	0.39
Milk fat	0.86	0.94	0.94	0.97	0.056	0.16	0.15	0.53
Milk protein	0.69	0.73	0.72	0.73	0.037	0.51	0.33	0.47
Milk lactose	0.92	0.94	0.94	0.91	0.042	0.82	0.85	0.28
CN	0.54	0.59	0.57	0.58	0.033	0.68	0.09	0.38
Milk composition (%)								
Fat	4.48	4.55	4.55	4.79	0.125	0.14	0.14	0.40
Protein	3.48 ^b	3.52 ^{ab}	3.49 ^b	3.64 ^a	0.068	0.21	0.09	0.30
Lactose	4.66	4.54	4.57	4.55	0.041	0.20	0.06	0.18
CN	2.73 ^b	2.87 ^{ab}	2.74 ^b	2.91 ^a	0.084	0.656	0.01	0.72

^{a–c}Values within rows followed by different superscripts differ ($P < 0.05$).

¹G– = grass silage-based TMR without supplementary HMBi, G+ = grass silage-based TMR with supplementary HMBi, C– = corn silage-based TMR without supplementary HMBi, C+ = corn silage-based TMR with supplementary HMBi.

²UFL = unit of energy for lactation.

ferent ($P > 0.05$) from those offered other treatments. Similarly, animals offered C+ had a greater concentration of CN in the milk than animals offered C– ($P = 0.04$) and G– ($P = 0.03$). Milk CN concentrations from animals offered G+ were not different ($P > 0.05$) than those from animals offered other treatments.

Nitrogen Balance

The effect of forage source and supplementary HMBi on N balance is presented in Table 4. Nitrogen intake ranged from 0.42 kg/d to 0.46 kg/d and was higher for animals offered CS-based diets ($P = 0.05$) than for those offered GS-based diets. Nitrogen intake was greater for animals offered C– than animals offered G– ($P = 0.02$) and G+ ($P < 0.01$). Feed N intake for animals offered C+ was not different ($P > 0.05$) from that for animals offered other treatments. The supply of PDI was also greater in animals offered CS-based diets ($P = 0.03$) when compared with those offered GS-based diets.

No effect was found ($P > 0.05$) of forage source or supplementary HMBi on the excretion of N in feces, urine, or milk and, therefore, diet had no effect ($P > 0.05$) on the efficiency of N use, calculated as N output in milk divided by N intake in feed. The contribution of PDI to milk production ranged from 1.06 kg/d to 1.09 kg/d and was not affected ($P > 0.05$) by dietary treatment. The balance of PDI supplied by the diet over that required for production and maintenance was higher for animals offered CS-based diets ($P = 0.02$) than for those offered GS-based diets.

Animals offered CS-based diets had higher urea levels in blood plasma (PUN; $P < 0.01$) when compared with animals offered GS-based diets. Similar effects of CS were noted for MUN ($P = 0.03$). No effect was observed ($P > 0.05$) of HMBi supplementation on urea N levels in plasma, milk, or urine. Animals offered G+ had a lower levels of PUN than those offered C– ($P < 0.01$) and C+ ($P < 0.01$) but not G– ($P = 0.15$). Plasma urea N concentrations were higher in animals offered C+ than in animals offered G– ($P < 0.01$) but not C– ($P = 0.21$). An interaction existed ($P = 0.06$) between forage and HMBi for PUN concentration. Animals offered C+ had higher concentrations of MUN than those offered G– ($P = 0.06$) and G+ ($P = 0.06$). Concentrations of MUN from animals offered C– were not different from those from animals offered other treatments ($P > 0.05$). Concentrations of urinary urea nitrogen (UUN) were greater from animals offered CS-based diets ($P < 0.01$) than from animals offered GS-based diets. Daily excretion of urea (g/d) was greater from animals offered CS-based diets ($P = 0.01$) when compared with those offered GS-based diets. Urea output was lower from animals offered G+ than from animals offered C– ($P = 0.06$) and C+ ($P = 0.06$). Urea output from animals offered G– was not different from those offered other treatments ($P > 0.05$). Urea output was lower from animals offered G+ than from animals offered C– ($P < 0.01$) and C+ ($P = 0.06$). Urea output from animals offered G– was not different from that of animals offered other treatments ($P > 0.05$). A positive linear relationship was observed ($P = 0.01$; $R^2 = 0.30$) between urea N excretion and feed N intake (Equation 1). Animals

Table 4. The effect of forage source and supplementary isopropyl ester of 2-hydroxy-4-methylthio butanoic acid (HMBi) on nitrogen balance

Item	Dietary treatment ¹				SEM	Significance		
	G–	G+	C–	C+		Forage	HMBi	Interaction
Intake (kg/d)								
Feed N	0.42 ^{bc}	0.42 ^c	0.47 ^a	0.45 ^{ab}	0.018	<0.01	0.36	0.59
PDI ²	1.71 ^b	1.71 ^b	2.05 ^a	2.03 ^a	0.082	<0.01	0.81	0.79
N output (kg/d)								
Fecal N	0.188	0.184	0.198	0.203	0.015	0.24	0.95	0.71
Urine N	0.123	0.123	0.138	0.124	0.008	0.21	0.26	0.32
Milk N	0.101	0.106	0.106	0.106	0.005	0.46	0.42	0.49
PDI milk ³	1.028	1.092	1.080	1.090	0.055	0.51	0.33	0.47
ENU ⁴ (%)	23.66	24.48	22.78	23.87	1.067	0.30	0.19	0.74
PDI balance ⁵ (kg/d)	0.341 ^b	0.291 ^b	0.558 ^a	0.558 ^a	0.071	<0.01	0.70	0.70
Urea-N (mmol/L)								
Plasma	1.87 ^{bc}	1.56 ^c	2.21 ^{ab}	2.47 ^a	0.157	<0.01	0.88	0.06
Milk	2.24 ^b	2.25 ^b	2.49 ^{ab}	2.58 ^a	0.131	0.02	0.68	0.71
Urine	92.3 ^b	85.3 ^b	124.3 ^a	122.4 ^a	5.098	<0.01	0.23	0.49
Urine urea output (g/d)	36.6 ^{ab}	33.7 ^b	41.6 ^a	38.9 ^a	2.15	0.01	0.13	0.93
Creatinine N (g/d)	8.26 ^a	7.81 ^{ab}	7.32 ^b	6.60 ^b	0.351	<0.01	0.09	0.39
Allantoin N (g/d)	9.80	9.19	6.85	9.03	1.143	0.12	0.42	0.18
Allantoin N:creatinine N	1.29	1.19	1.06	1.37	0.265	0.88	0.57	0.25
Rumen NH ₃ -N (mg/L)	64.39 ^a	65.38 ^a	58.83 ^b	58.00 ^b	2.575	0.01	0.98	0.55
Excretion (kg/d)								
Feces	45.36	44.88	47.80	47.16	1.637	0.04	0.60	0.94
Urine	14.80 ^a	14.63 ^a	12.34 ^b	11.82 ^b	0.359	<0.01	0.29	0.57
Total slurry	60.16	59.51	60.14	58.91	1.776	0.82	0.46	0.83
Slurry DM (%)	7.84 ^b	8.25 ^b	9.07 ^a	9.53 ^a	0.249	<0.01	0.08	0.83

^{a-c}Values within rows followed by different superscripts differ ($P < 0.05$).

¹G– = grass silage-based TMR without supplementary HMBi; G+ = grass silage-based TMR with supplementary HMBi; C– = corn silage-based TMR without supplementary HMBi; C+ = corn silage-based TMR with supplementary HMBi.

²Protein truly digested in the small intestine.

³PDI milk = milk protein content (g/kg) × milk output (kg/d) × 1.5 (O'Mara, 1996).

⁴ENU = efficiency of N use [milk N output (kg)/feed N intake (kg) × 100].

⁵PDI balance = PDI intake – (PDI maintenance + PDI milk).

offered GS-based diets had greater urinary creatinine excretion ($P < 0.01$) when compared with animals offered CS-based diets. Urinary creatinine N excretion was greater from animals offered G– than from those offered C– ($P = 0.04$) and C+ ($P < 0.01$) but not G+ ($P = 0.25$); G+, C–, and C+ were not different ($P > 0.05$). Allantoin N and the ratio of allantoin N to creatinine N were not affected by dietary treatment ($P > 0.05$). Rumen fluid NH₃-N (mg/L) was higher for animals offered GS-based diets ($P = 0.01$) than for those offered CS-based diets.

$$\text{Urea N (g/d)} = 11.26 (\pm 7.39) + 59.87 (\pm 16.61) \times \text{feed N (kg/d)}. \quad [1]$$

Animals offered GS-based diets excreted more urine ($P < 0.01$) when compared with animals offered CS-based diets. However, there was no effect of forage source or HMBi supplementation on either feces output (kg/d) or total slurry (combined urine and feces) output (kg/d; $P > 0.05$). Slurry DM was higher with CS-based diets ($P < 0.01$) when compared with GS-based diets.

DISCUSSION

DMI, Milk Production, and Milk Composition

The DM, starch content and DMD of the CS used in this experiment (Table 1) were lower than those reported in recent experiments comparing CS and GS (Mulligan et al., 2002; Burke et al., 2007; Cooke et al., 2009), indicating that the crop was of poorer quality than that used in previous experiments. Additionally, the GS used in this study was of poor quality for lactating dairy cows containing low levels of CP, UFL, and DMD. However, ME and DMD of GS were comparable with values reported in an older study by Phipps et al. (1992). Despite the differences in forage quality between experiments, the observation of improved DMI when CS is included at the expense of GS in the forage DM is consistent with observations made by O'Mara et al. (1998), Burke et al. (2007), and Cooke et al. (2009) when portions of GS are replaced by CS in the forage DM. The increase in DMI with CS-based diets in this study can be accounted for by the higher DM in CS-based diets than in GS-based diets. A greater difference in DMI may be achieved through further substitution

of GS with CS as reported in O'Mara et al. (1998) and Cooke et al. (2009), providing other nutrients such as CP do not limit intake as reported in Phipps et al. (1992).

Increases in DMI when replacing GS with CS are commonly associated with increased energy intake and, subsequently, increased MY, provided diet DMD is not decreased in high-CS diets (O'Mara et al., 1998). Although animals offered CS-based diets had greater energy intake than those offered GS-based diets, the observed decrease in DMD for CS-based diets (Table 2) may have decreased the potential for improvements in MY. The lack of response of MY to supplementary HMBi is consistent with previous observations made by Broderick et al. (2008) and Phipps et al. (2008), suggesting that, at the production levels reported herein, animals did not require additional methionine in the diet. Lysine is also an important AA for milk production (NRC, 2001). Broderick et al. (2008) noted that supplementation with methionine to diets containing a higher CP (16.1%) resulted in a greater MY when compared with lower-CP diets (14.8%), suggesting that other AA become limiting at the lower dietary CP. Although the diets used in the current experiment were calculated to contain adequate lysine to support the production levels reported herein, it is possible that a further response to MY may have been limited by other AA, consistent with observations of Phipps et al. (2008). The marginal improvement in the yield of milk solids with HMBi supplementation is a result of numeric increases in milk fat and protein yields.

Substituting GS with CS in this experiment had little effect on milk composition. Previous authors have reported increases in milk protein concentration where CS replaces GS in the forage DM (Mulligan et al., 2002; Burke et al., 2007; Cooke et al., 2009). It is quite likely that the increase in milk protein in those experiments was driven by additional starch in the diets containing high portions of CS. Starch acts as a source of FOM for microbial protein synthesis and, subsequently, increases the quantity of microbial CP reaching the small intestine (Jarrige, 1989). Additionally, the increase in propionate production associated with starch fermentation increases glucose production (Rook and Balch, 1961), which is known to increase AA availability and uptake in the mammary gland (Rulquin et al., 2004; Rius et al., 2010). The high inclusion of rolled barley grain in GS-based diets used in this study decreased the differences in starch composition between diets and, therefore, the potential for an improvement in milk protein concentration. Animals that were supplemented with HMBi showed a marginal improvement in protein and CN concentrations with a concurrent increase in CN yield. These improvements are consistent with previ-

ous reports where animals were supplemented with a methionine source (Varvikko et al., 1999; Phipps et al., 2008; Robinson, 2010), suggesting an improvement in the AA profile available to the mammary gland.

Nitrogen Balance

The conversion of ingested feed N into milk N is poor in dairy cows, with up to 75% of ingested feed N being recovered in the feces and urine (Olmos Colmenero and Broderick, 2006). In the current experiment, the recoveries of ingested feed N in the feces, urine, and milk were 44, 29, and 24%, respectively, across dietary treatments. The increase in N intake with CS-based diets is consistent with the increase in DMI with these diets. However, as the response of milk N output to increased N intake is poor (Mulligan et al., 2004), the increase in N intake did not result in an increase in milk N out. As supplementary HMBi did not improve milk protein yield, supplementary HMBi had no effect on milk N output.

The observed ratio of fecal N to urinary N (1.6:1.0) in this study is consistent with feeding situations where dietary CP is decreased. Previous researchers (Castillo et al., 2001; Mulligan et al., 2004; Olmos Colmenero and Broderick, 2006), have observed decreases in urinary N with relatively little change in fecal N as dietary N intake is decreased, thus increasing the ratio of fecal N to urinary N. In a study where dietary CP was decreased (19.4 vs. 13.5%), Olmos Colmenero and Broderick (2006) observed an increase in the ratio of fecal N to urinary N (1.7:1.0 vs. 0.82:1.0). Fecal N, consisting largely of undigested feed N and endogenous N (McDonald et al., 2002), is less volatile than urinary N. Therefore, despite the absence of a significant dietary effect on the ratio of fecal N to urinary N in the present experiment, the potential for a decrease in gaseous N loss with diets of this CP may be environmentally advantageous.

Decreasing NH_3 loss to the atmosphere through decreasing urinary urea N excretion is also of environmental interest (Bussink and Oenema, 1998). In ruminant species, the principal sources of urea are NH_3 -N produced in the rumen and AA catabolism in the liver (DePeters and Ferguson, 1992). The higher concentrations of NH_3 -N in the rumen fluid of animals offered GS-based diets is a reflection of the higher level of rumen-degradable N available in GS compared with CS. However, high levels of starch inclusion, acting as a source of fermentable carbohydrate (Herrera-Saldana et al., 1990), and low levels of fermentable N in the diets offered in this study may have decreased the potential for NH_3 -N loss from the rumen. Given that animals offered CS-based diets had greater urea concentrations in

the blood, milk, and urine, it is likely that the principal source of urea in CS-based diets was a greater supply of PDI to the intestine not utilized in the synthesis of milk protein and, subsequently, catabolized by the liver. The numeric decrease in urinary urea concentration in animals supplemented with HMBi is consistent with the observed improvement in milk protein concentration.

Overall, concentrations of urea in the plasma and milk reported herein were lower than those reported in Olmos Colmenero and Broderick (2006), despite CP levels being similar (133 vs. 135 g/kg of DM). The diurnal pattern of urea concentration in plasma (Gustafsson and Palmquist, 1993) and differences in milk urea analysis (infrared vs. colorimetric; Broderick, 2003) may partially account for the disparity between these studies. However no indication exists that the animals used in this experiment were restricted by dietary CP, as MUN was above the 1.4 mmol/L lower threshold suggested by Vérité et al. (1996). The increase in urinary allantoin excretion from animals offered GS-based diets suggests a greater flow of microbial N to the small intestine with these diets (Broderick, 2003).

Excretion of urea N in this experiment was positively related to N intake and, as such, was greater in animals offered CS-based diets when compared with those offered GS-based diets. Increasing dietary N intake without a corresponding increase in milk N output is undesirable and results in excessive urea N excretion and potential losses to the environment as NH_3 . Furthermore, the slurry from animals offered CS-based diets had a higher DM content compared with GS-based diets. Decreasing slurry DM content improves infiltration rate into the soil, which, in turn, will decrease the potential for N loss as NH_3 (Sommer and Olesen, 1991; Bussink and Oenema, 1998).

CONCLUSIONS

Neither forage source nor supplementary HMBi altered N output in the current study. Replacing GS with CS in the forage DM resulted in increased DMI and N intake. However, no subsequent improvements in milk N output occurred and, consequently, urinary urea excretion was higher with CS-based diets. Animals did not appear to be limited by the supply of available methionine at the production levels reported herein. Therefore, improvements in milk yield and milk N output were not observed where diets containing 13.5% CP were supplemented with HMBi. Improvements in the DMD, UFL, and CP content of GS offered and the use of higher-yielding cows may alter these results, highlighting an area where further investigation is required.

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