Effects of crude protein level in concentrate supplements on animal performance and nitrogen utilization of lactating dairy cows fed fresh-cut perennial grass

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ABSTRACT

Nitrogen pollution of air and ground water from grazing cattle is of increasing concern. Although several studies have investigated mitigation strategies for nitrogen output from dairy cows fed conserved forages and concentrates, similar research on fresh-cut grass in addition to production parameters is limited. The current study, using 3 dietary treatments and incorporating 2 genotypes, was designed to evaluate the effects of concentrate crude protein (CP) levels on animal production and nitrogen utilization efficiency (NUE) in lactating dairy cows. Twelve multiparous cows (6 Holstein and 6 Holstein × Swedish Red) were used in a changeover study with three 25-d periods and 3 diet treatments. Low, medium and high CP concentrate [14.1, 16.1, and 18.1%, respectively, dry matter (DM) basis] diets were fed at 32.8% DM intake combined with good-quality zero-grazed perennial ryegrass (18.2% CP, DM basis). Each period consisted of an adaptation phase (18 d) housed as a single group, a 1-d adaptation phase in individual stalls, and a 6-d measurement phase with feed intake and feces, urine, and milk output recorded. We observed no significant interaction between cow genotype and concentrate CP level on any animal performance or NUE parameter. Total DM intake, milk yield and composition, and NUE were not affected by dietary treatment. However, increasing concentrate CP level increased (1) N intake by 42 g/d and excretion in urine and manure by 38 and 40 g/d, respectively, and (2) the ratio of urine N over manure N. Feeding high CP rather than low CP concentrate increased milk urea N (MUN) content by 3.6 mg/dL and total

MUN output by 1.08 g/d. Crossbred cows had lower grass DM intake, total DM intake, total N intake, and energy-corrected milk yield. However, cow genotype had no significant effect on NUE or MUN parameters. Equations have been developed to predict urine N excretion using MUN output as a sole predictor or in combination with dietary CP level. The present study indicated that when grazing cows are fed good-quality pasture, feeding concentrates with a protein content as low as 14.1% may not negatively affect productivity. In addition, reducing concentrate CP concentration may be successful in reducing the urinary N excretion of lactating dairy cattle on pasture-based systems, but further research is needed to investigate the long-term effects of supplementary concentrate CP content on milk production.

Key words: dairy cow, concentrate protein content, fresh grass, milk production, nitrogen utilization

INTRODUCTION

Greenhouse gas emissions from livestock production systems, specifically ruminants, are a major source of environmental concern. With normal bovine feeding practices, a large percentage of dietary protein is inefficiently used, leading to increased manure N output and resulting environmental, health (Butler, 1998), and economic implications. Excess N excretion from ruminants can be converted to many forms, such as ammonia, a major air pollutant; N₂O, a greenhouse gas; and nitrate, a water pollutant. The considerable variation in levels of N excretion in urine across a range of dietary treatments highlights the potential for alleviation (Castillo et al., 2000). Grasslands are the most economical feedstuff for dairy farmers in Northern and Western Europe (Peyraud and Delagarde, 2013). However, because controlling forage nutrient composition can prove difficult,

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a feasible mitigation option for improving nitrogen utilization efficiency (**NUE**) may be to reduce the CP content in concentrate feeds. This may be possible in pasture-based systems as opposed to indoor systems on silage-based diets because pasture often possesses a CP content in excess of or close to 20% on a DM basis (Kavanagh et al., 2003), a value considerably greater than that typically found in conserved forage. Hence, it is vital that N partitioning be assessed in all commonly used farming practices to reduce pollution and maintain herd health in a cost-effective manner across dairy production systems. Previous studies have shown improved NUE, in particular reduced urinary N excretion, as a result of reduced concentrate CP levels (Castillo et al., 2000; Marini and Van Amburgh, 2005; Burke et al., 2008). However, whether improved NUE and N partitioning, in addition to production responses, can be achieved using low CP concentrates in a fresh-cut grass-based diet has not yet been determined.

Evidence also points to a genetic effect on N metabolism (Pareek et al., 2007; Beecher et al., 2014), although to a lesser extent than dietary CP content (Huhtanen et al., 2015). It is well documented that MUN is used to monitor feed management practice, specifically excess dietary CP, and has been suggested as an indicator for urinary N excretion (Jonker et al., 1998; Kauffman and St-Pierre, 2001). Previous literature has found the relationship between urinary N and MUN concentration may be subject to genetic influence (Kauffman and St-Pierre, 2001), with significant differences found between Holstein and Jersey cows. Some of this variation may be explained by milk yield and BW (Huhtanen et al., 2015) or as a result of genetic variation in urea transporters in the kidney and across the rumen epithelium, with different alleles resulting in increased or reduced activity (Aguilar et al., 2012). Conversely, some trials found no evidence of a genetic effect on N utilization (Zou et al., 2016) or MUN concentration (Carlsson et al., 1995). Swedish Red is a high-producing breed in common use in Northern Europe that has been crossed with Holsteins to improve fertility, udder health, and longevity (Heins and Hansen, 2012), resulting in greater projected lifetime profit and profit per cow-day than the Holstein breed (Heins et al., 2012). Because Holstein and Swedish Red represent important bovine breeds for milk yield and solids output, a comparison between Holstein and Holstein × Swedish Red crossbreds would be suitable for examining the genetic and physiological effects on variation of N partitioning in dairy cattle.

The objective of the present study was (1) to investigate the effects of animal genetics and varying concentrate CP content on milk production levels in combination with NUE and N partitioning parameters and (2)

to develop linear and multiple relationships to estimate MUN and urinary N outputs for lactating dairy cows on similar diets to those offered in the present study using readily available data at the farm level.

MATERIALS AND METHODS

All animal procedures in the present study were conducted under experimental license from the Department of Health, Social Services and Public Safety of Northern Ireland in accordance with the Animal (Scientific Procedures) Act (Home Office, 1986).

Experimental Design

The current study was conducted during the 2014 grazing season at Agri-Food and Biosciences Institute (Hillsborough, Northern Ireland, UK), using 6 pure Holstein and 6 crossbred (50:50 Holstein × Swedish Red) cows fed fresh-cut grass and 3 differing concentrate feeds in a 3-period (25 d/period) changeover design study. Cows from each genotype were blocked into 3 groups of 2 cows based on milk yield, BW, and lactation stage, and were then randomly allocated to one of the 3 dietary treatments. The mean milk yield, BW, and DIM at the start of the trial were 26 ± 4.9 kg/d, 550 ± 39.9 kg, and 119 ± 20.5 d, respectively. The diet treatments were a low CP concentrate (**LCP**, 14.1%), a medium CP concentrate (**MCP**, 16.1%) and a high CP concentrate (**HCP**, 18.1%) on a DM basis offered at 35% DMI, in combination with fresh-cut perennial ryegrass offered at 65% DMI. Each experimental period consisted of (1) an initial 18-d feed adaptation phase, where cows were housed as a single group with individual feed intake recorded; (2) a 1-d adaptation phase in individual stalls; and (3) a 6-d digestibility unit phase, with daily recording of feed intake and total collection of feces, urine, and milk outputs.

The LCP and HCP concentrates were formulated separately; both contained the same feed ingredients and similar chemical composition (with the exception of CP content). The MCP concentrate was then produced by mixing the LCP and HCP concentrates in a 1:1 (wt/wt) ratio. The ingredients and chemical composition of LCP and HCP concentrates are presented in Tables 1 and 2, respectively. Half of the daily concentrate rations were offered at morning milking (0700 h) and half at afternoon milking (1500 h), and fresh-cut grass, harvested with a Haldrup 1500 (Plot Combine, Haldrup, Ilshofen, Germany) from a single sward, was offered at 1000 h each morning ad libitum. Herbage received a primary cut in April 2014 and was subsequently harvested at regrowth intervals according to month (increasing from 22 to 30 d from June to Sep-

Table 1. Concentrate ingredient composition (g/kg of DM)¹

Ingredient	LCP	HCP
Corn	246	220
Wheat feed	140	135
Corn gluten	140	135
Soy hulls	140	135
Palm kernel expeller	110	110
Sugar beet pulp	45	0
Sunflower kernel	66	60
Soybean meal	0	80
Rapeseed extract	0	27
Molaferm	70	50
Pure palm oil	7	7
Limestone flour	14	19
Salt	8.5	9.4
Calcined magnesite	8.8	8.6
Trace elements and vitamins ²	4.0	4.0

¹LCP = low CP concentrate (14.1%, DM basis); HCP = high CP concentrate (18.1%, DM basis).

²Trace elements and vitamins consisted of 25 IU/kg of vitamin E, 5 mg/kg of I, 0.6 mg/kg of Se, 30 mg/kg of Cu, 50 mg/kg of Mn, and 100 mg/kg of Zn, 9,000 IU/kg of vitamin A, and 2,000 IU/kg of vitamin D₃.

tember), generating grass of a similar quality to that under commercial management. Grass in the sward consisted of a 3-yr re-seed of 'Aberstar', 'Aberzest', and 'Alice' varieties, sown in a ratio of 8:5:1, respectively, and paddocks had not been grazed since the end of the previous grazing season (November 2013). Post-harvesting fertilization was implemented within 3 d at 35 kg of N/ha. The temperature of the fresh-cut grass was monitored throughout the study to minimize the risk of nutrient degradation by plant proteases (Callis, 1995). Animals had free access to water throughout the experiment. Concentrate offered was calculated for individual animals as 35% total DMI using the previous 7-d running average of ad libitum forage intake.

Measurements

Body weight was recorded before and after the digestibility unit phase. Daily herbage intakes and re-

fusals were recorded, sampled, and analyzed for oven DM at 85°C during the 6-d measurement phase at the end of each period. Fresh herbage samples were dried in an oven at 60°C for 72 h (Ruiz et al., 2001; Jiao et al., 2014), milled through a 0.8-mm screen and analyzed for ADF, NDF, ash, gross energy (**GE**), N, and water-soluble carbohydrates (**WSC**) content on a daily basis. Concentrate samples (200 g) were taken 4 times per week and dried for 48 h at 100°C according to AOAC (1980; official method 14.063). Samples were then composited, milled through a 0.8-mm screen, and analyzed for weekly determination of DM, ADF, NDF, ash, GE, starch, and N concentrations. Feces and urine outputs were weighed, recorded, and sampled separately as a percentage (5%) of total fecal output (by weight) and urine output (by volume) for the 6-d collection phase in the digestibility units. Daily urine and fecal samples were stored at 4°C after collection, and 3-d samples were pooled for analysis. Samples were thoroughly mixed, and a representative sample was obtained for fresh analysis of N content for feces and urine, according to the method in Jiao et al. (2013). A subsample of the bulked 3-d feces samples was dried at 85°C for subsequent DM, ADF, NDF, and ash analysis, as described by Cushnahan and Gordon (1995). To prevent ammonia volatilization from urine samples during the 24-h collection, sulfuric acid solution (50% H₂SO₄) was added to the urine canisters before collection to achieve a pH between 2.0 and 4.0 (Freudenberger et al., 1994). Milk samples of 2% volume were collected twice daily, bulked for 3-d phases, and frozen (−20°C) until analysis. Milk samples were analyzed by Milkoscan (Foss Electric, Hillerød, Denmark) for fat, protein, and lactose. Contents of MUN were measured using the QuantiChrom urea assay kit (DIUR-500) after a deproteinization step (BioAssay Systems, Hayward, CA). Analysis of milk GE was performed according to the method described by Jiao et al. (2013). Determination of GE, N (grass and concentrate only), and ash was performed as described previously by Cushnahan and

Table 2. Chemical composition (g/kg of DM, unless otherwise stated) of dietary components used in the present experiment

Component	Grass			Concentrate ¹	
	July	August	September	LCP	HCP
DM (g/kg)	154	147	161	898	898
Ash	100	94	94	89	91
CP	18.8	17.8	18.3	14.1	18.1
Gross energy (MJ/kg of DM)	18.7	18.7	18.4	18.0	18.1
NDF	490	454	440	369	369
ADF	239	234	222	189	187
Starch	—	—	—	232	211
Water-soluble carbohydrates	130	171	184	—	—

¹LCP = low CP concentrate (14.1%, DM basis); HCP = high CP concentrate (18.1%, DM basis).

Gordon (1995). For analysis of grass, concentrate, and milk concentrations of GE a Parr 6300 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL) was used. Total N content was determined on a DM basis for grass and concentrate, and on a fresh basis for feces and urine, using a Vario Max CN (Elementar, Hanau, Germany) and a Kjeltec 2400 analyzer (Foss Tecator AB, Höganäs, Sweden), respectively. Ash in grass, concentrate, and feces was determined by incineration in a muffle furnace (Vecstar, Derbyshire, UK) at 550°C for approximately 10 h (AOAC, 1990). Ash-corrected concentrations of ADF and NDF were determined sequentially using a Fibertec fiber analyzer (Foss Electric, Hillerød, Denmark). The NDF was assayed using sodium sulfite and α -amylase, as described by Van Soest et al. (1991). The total starch content of concentrate was measured using a total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland; McCleary et al., 1994). The WSC content of grass was determined spectrophotometrically using anthrone in sulfuric acid and the Technicon Autoanalyzer (Technicon Corp., New York, NY; Thomas, 1977).

Statistical Analysis

Energy-corrected milk yield was calculated as milk energy output (milk yield multiplied by measured milk energy concentration) divided by milk energy content in 1 kg of standard milk (40 g/kg fat, 32 g/kg protein, and 48 g/kg lactose) using the equation of Tyrrell and Reid (1965). Experimental data were analyzed using the Genstat statistical package (VSN International, 2013). All variables were analyzed using the linear mixed model methodology with REML estimation (Gilmour et al., 1995). In the analysis, which was based on individual animal data, cow and date (of entry to collection phase) were fitted as random effects, and genotype and treatment as fixed effects. Orthogonal polynomial contrasts (linear and quadratic) were used to examine treatment effect on response variables. The significance of fixed effects was assessed by comparing an F-statistic against an F-distribution. Residuals showed no deviation from normality. The differences between treatments, genotypes, and interactions were assessed and declared as nonsignificant at $P > 0.05$ and significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$. A REML analysis was also performed to develop a range of linear and multiple relationships to estimate MUN and urine N outputs using the method previously described by Stergiadis et al. (2015). In brief, linear regression relationships were developed where the responses were MUN output, MUN concentration, and urine N output, and the explanatory variables were N intake,

dietary CP content, and MUN output, respectively. A multiple linear regression was also developed to predict urine N output using MUN and dietary CP content as explanatory variables. The potential random effects of cow and date of entry were removed in all equations. The Wald statistic was used to evaluate the significance of the fixed terms. For all equations, a pseudo- R^2 was also generated, which describes the squared correlation of the response and the fitted values, to represent the amount of variability explained.

RESULTS

The effect of the main factors was significant on several feed/nutrient intake, production, and NUE parameters, but we observed no significant interaction between cow genotype and dietary treatment. Focus in the results and discussion sections will be on main treatment effects.

Diet Composition

The chemical composition of individual dietary components is given in Table 2. Grass NDF and ADF content both decreased and WSC content increased from July to September; but we observed no seasonal variation for ash, CP, or GE contents of grass. The perennial ryegrass offered in the present experiment contained an average DM of 154 g/kg; GE of 18.6 MJ/kg DM; CP of 18.2% DM; and 95.4, 456, 231, and 167 g/kg DM for ash, NDF, ADF, and WSC respectively. The chemical composition of the 3 concentrates was very similar, except for the CP content, which resulted in total dietary CP levels for the LCP, MCP, and HCP diets of 16.9, 17.6, and 18.3% DM, respectively.

Feed Intake and Milk Production

The effects of concentrate CP contents and cow genotype on feed intake and animal characteristics and production parameters are displayed in Table 3. On average, animal diets were composed of (DM basis) 67.2% fresh grass and 32.8% concentrate feed. Concentrate CP levels had no significant effect on voluntary feed intake or on milk production and composition. In contrast, cow genotype had significant effect on feed intake, animal characteristics, and milk production and composition parameters. Holstein cows had significantly higher grass intake (+6.7%) and DMI (+5.4%) than crossbred cows. Holstein cows produced significantly higher yields of ECM (3.6 kg/d or +14.1%) and had significantly higher milk lactose content (+3.4%) but lower milk protein content (−10.5%).

Table 3. Effect of concentrate CP level and cow genotype on animal, feed intake, and production parameters

Parameter	Concentrate CP level			P-value ¹		Cow genotype			SEM	P-value
	Low	Medium	High	SEM	L	Q	Holstein	Crossbred ²		
Animal characteristics										
BCS	2.37	2.30	2.34	0.038	0.46	0.22	2.29	2.39	0.044	0.12
BW (kg)	579	582	571	15.2	0.32	0.43	583	573	20.8	0.74
Feed intake (kg of DM/d)										
Grass	13.8	14.1	14.1	0.37	0.30	0.24	14.4	13.5	0.32	0.009
Concentrate	7.0	7.0	6.9	0.16	0.61	0.40	7.0	6.9	0.16	0.30
Total DM	20.7	21.0	21.0	0.47	0.57	0.21	21.5	20.4	0.43	0.019
Production										
Milk yield (kg/d)	25.8	26.5	26.7	1.36	0.55	0.93	28.5	24.2	1.49	0.070
ECM yield (kg/d)	27.1	27.1	27.6	1.00	0.56	0.62	29.1	25.5	0.81	0.007
Milk fat content (g/kg)	42.0	41.5	41.8	1.48	0.86	0.36	40.5	43.0	2.01	0.39
Milk protein content (g/kg)	36.1	36.2	36.4	1.09	0.99	0.88	34.2	38.2	1.18	0.030
Milk lactose content (g/kg)	44.7	45.0	45.0	0.39	0.091	0.82	45.7	44.2	0.38	0.016

¹Probability of a linear (L) or quadratic (Q) effect of concentrate CP level in the diet.²Crossbred cows were crosses between Holstein and Swedish Red.

N Partitioning and Utilization

The effects of concentrate CP content and cow genotype on N intake, outputs, and utilization variables are displayed in Table 4. Intake of total and digestible N increased linearly with increasing concentrate CP content. Cows fed the HCP diet consumed 42 g/d more total N) and 37 g/d more digestible N than cows fed the LCP diet. Feeding LCP concentrate significantly and linearly reduced urine N excretion compared with feeding HCP concentrate (−38 g/d). Excretion of manure N increased linearly with increasing concentrate CP content (+40 g/d for cows fed the HCP diet compared with those fed the LCP diet). Dietary treatment exerted no significant effect on N outputs in feces or milk, retained N, and several NUE parameters (proportion of N intake excreted in feces, urine, manure, milk, and the ratio of retained to digested N). In fact, we observed a shift in N excretion from urine to feces when expressed relative to manure N; the proportion of urine N was significantly decreased and the proportion of feces N was significantly increased in cows fed the LCP diet compared with cows fed the HCP diet.

Compared with crossbred cows, Holstein cows had significantly higher intakes of total N (+25 g/d) and digestible N (+19 g/d), but genotype had no significant effect on any NUE variable.

MUN Output

Milk urea N output values are shown in Table 5. We found that MUN output increased linearly with increasing concentrate CP content, so that MUN values in cows fed the HCP diet were on average 1.08 g/d higher than cows fed the LCP diet. We also found that MUN concentrations declined linearly with decreasing concentrate CP content (−1.6 and −3.6 mg/dL for cows offered MCP and LCP compared with HCP diets, respectively). However, concentrate CP level had no significant effect on MUN output when expressed as a proportion of total N intake or digestible N intake. The effect of cow genotype on MUN excretion, concentration, or proportion to total N or digestible N intakes was not significant.

Estimation of MUN and Urine N Output

When linear and multiple relationships for estimating urine N output and MUN output and concentration were developed, the explained variation was higher for prediction of MUN parameters (Table 6). The effect of (1) N intake and dietary CP content for the prediction of MUN output and MUN concentrations, respectively, and (2) MUN and dietary CP content for the prediction

Table 4. Effect of concentrate CP level and cow genotype on N intake and output and N utilization efficiency parameters

Item	Concentrate CP level				P-value ¹			Cow genotype		
	Low	Medium	High	SEM	L	Q		Holstein	Crossbred ²	SEM
N intake/output (g/d)	543	572	585	16.6	<0.001	0.17		579	554	12.6
Total dietary N intake	358	382	395	13.7	<0.001	0.038		388	369	12.7
Digestible N intake	187	187	188	6.4	0.86	0.81		190	185	6.0
Feces N	193	208	231	10.8	0.004	0.63		220	202	10.6
Urine N	380	394	420	12.8	0.017	0.65		409	387	12.5
Manure N	149	154	156	5.5	0.41	0.89		157	149	6.2
Milk total N	144	149	150	5.2	0.54	0.91		151	145	5.6
Milk protein N	15.4	22.3	7.9	15.54	0.61	0.17		14	17	15.6
Retained N										
N utilization (g/g)										
Feces N/N intake	0.345	0.332	0.327	0.0118	0.088	0.67		0.334	0.336	0.0120
Urine N/N intake	0.356	0.363	0.402	0.0210	0.054	0.46		0.380	0.367	0.0214
Manure N/N intake	0.701	0.694	0.727	0.0253	0.29	0.35		0.711	0.703	0.0270
Milk total N/N intake	0.274	0.271	0.270	0.0085	0.63	0.88		0.272	0.271	0.0076
Milk protein N/N intake	0.265	0.262	0.260	0.0080	0.51	0.90		0.265	0.262	0.0074
Retained N/N intake	0.024	0.036	0.004	0.0288	0.43	0.12		0.017	0.026	0.0289
Feces N/Manure N	0.497	0.478	0.452	0.0157	0.007	0.77		0.469	0.481	0.0158
Urine N/Manure N	0.503	0.522	0.548	0.0157	0.007	0.77		0.531	0.519	0.0158

¹Probability of a linear (L) or quadratic (Q) effect of concentrate CP level in the diet.

²Crossbred cows were crosses between Holstein and Swedish Red.

Table 5. Effect of concentrate CP level and cow genotype on MUN contents, excretion, and ratios to N intake

Item	Concentrate CP level				P-value ¹			Cow genotype		
	Low	Medium	High	SEM	L	Q		Holstein	Crossbred ²	SEM
MUN (g/d)	4.85	5.35	5.93	0.476	0.016	0.86		5.82	4.89	0.473
MUN content (mg/dL)	18.9	20.9	22.5	1.23	<0.001	0.75		20.7	20.7	1.26
MUN/N intake	0.0090	0.0094	0.0103	0.00087	0.093	0.41		0.0101	0.0090	0.00087
MUN/digestible N intake	0.0141	0.0141	0.0157	0.00155	0.29	0.23		0.0155	0.0137	0.00156

¹Probability of a linear (L) or quadratic (Q) effect of concentrate CP level in the diet.

²Crossbred cows were crosses between Holstein and Swedish Red.

Figure 1. Relationship between MUN and urine N output for lactating dairy cows on diets of 2:1 fresh grass:concentrate ratio, as presented in equation [3] in Table 6.

of urine N output, were significant according to the Wald statistic, and all relations were positive. Figure 1 displays the positive relationship between urine N output (g/d) and MUN output (mg/d), as shown in equation [3] in Table 6.

DISCUSSION

The manipulation of concentrate CP concentration is commonly used to optimize rumen microbial activity and milk production for grazing and confined dairy production systems. Responses in NUE have been extensively evaluated in confined dairy cows offered grass silage, but such information may not be accurate for grazing cows, as the ensiling process can considerably alter the nutritive value of forage. Increases in the CP fraction A (NPN) at the expense of CP fraction B (true protein), rate of proteolysis, and VFA concentrations, as well as reductions in carbohydrate content, occur during ensiling. In addition, daily deviations in pasture CP content are more pronounced compared with conserved forage, which may also affect the ruminal protein-energy balance. The present study was designed to evaluate the effects of manipulation of concentrate CP

concentration on milk production and NUE of dairy cows offered fresh grass.

Diet Composition

Ryegrass used in the present study was typical for good-quality ryegrass (Ministry of Agriculture, Fisheries and Food, 1992). The WSC content of the fresh-cut grass increased between July and September, possibly due to longer grass regrowth intervals toward the end of the grazing season (Owens et al., 2008). Throughout the present experiment, good-quality ryegrass was offered, averaging 18.2% CP, 461 g/kg of NDF, and 162 g/kg of WSC. Consequently, animals consumed higher than expected levels of fresh grass in the measurement periods, leading to a marginally higher dietary forage proportion than the designed level (67.2% vs. 65% DM basis). These 2 factors in combination may have reduced the extent of the responses between treatments for some of the parameters.

Production Performance

Although concentrate feed was designed to be 35% DMI, the actual concentrate intake was 32.8% of total DMI due to the higher grass DMI (14.0 kg/d) in the digestibility units than in the housing cubicles (12.6 kg/d). The concentrate feed proportion was chosen to be representative of commercial practice in the UK (Ferris, 2007) and to be of a sufficient level to achieve significant differences in total dietary CP content across treatments. The results from the present study implied that feeding a concentrate of 14.1% CP when good-quality perennial ryegrass is grazed might sustain milk yield and milk quality in pasture-based systems. Previous studies found that offering concentrate of 15% CP to supplement grazing was associated with a decrease in milk yield of 2.9 kg/d compared with feeding a 19% CP concentrate (Whelan et al., 2012); low-protein diets (14–16% CP) also decreased production and tended to decrease milk protein content in corn and grass-clover silage-based diets (Alstrup et al., 2014). More recent studies have shown that concentrates with CP content

Table 6. Regression models for the prediction of MUN and urine N excreta from lactating dairy cows

Equation no.	Equation ¹	R ²
1	MUN output (g/d) = $-3.1_{(2.69)} + 0.015_{(0.0047)}$ N intake (g/d)	0.946
2	MUN content (mg/dL) = $-31.3_{(8.64)} + 0.295_{(0.0486)}$ diet CP content (g/kg of DM)	0.975
3	Urine N output (g/d) = $139.1_{(18.07)} + 0.0142_{(0.00316)}$ MUN (mg/d)	0.792
4	Urine N output (g/d) = $-144.4_{(72.32)} + 0.010_{(0.0028)}$ MUN (mg/d) + $1.74_{(0.432)}$ diet CP content (g/kg)	0.802

¹Values in subscript parentheses represent standard errors. The effects of all explanatory variables were significant according to the Wald statistic ($P < 0.05$). The potential random effects of cow and date were removed for all predicted variables.

as low as 14% might be fed to dairy cows without negative implications for milk production (Sinclair et al., 2014). A range of diet and animal factors could influence the effect of concentrate CP levels on the milk production of grazing cows, including milk production potential, stage of lactation, and forage quality (Moran, 2005; De Oliveira et al., 2010). In the present study, the high milk protein content observed across all treatments is generally considered indicative of a high-energy diet (Broderick, 2003), which may have been a result of the quality of grass offered. The results of the present study indicate that dairy cows grazing good-quality pasture can be offered low CP concentrates, resulting in a total dietary CP content of 16.9% DM with no negative effect on feed intake or milk production.

N Partitioning and Utilization

We observed that increasing concentrate CP levels in a predominantly fresh ryegrass diet supplemented with concentrate increased total intake of N and digestible N. Feces N values were less variable (144–246 g/d) than urine N values (112–302 g/d), and this result was similar to those observed in the literature (Ruiz et al., 2001; Lee et al., 2009; Kebreab et al., 2010). In the present study, the nonsignificant effect of concentrate CP concentration on feces N excretion was partially due to similar DMI between treatments, an influential factor in fecal N output. This finding may also indicate that the ammonia-N supply from the LCP diet was enough to meet the requirement of rumen microbial growth, and the excess supply of degradable N in the MCP or HCP diet was excreted in urine as urea. Indeed, we found that urine N outputs were significantly higher with the HCP diet. Compared with the LCP diet, the additional N intake in the HCP diet (42 g/d) was almost entirely excreted in urine (38 g/d), demonstrating the sensitivity of the correlation between urinary N excretion and supplementary concentrate N. Broderick and Reynal (2009) observed an increase of 96 g/d in urine N excretion associated with an increase in dietary CP intake from 15.1 to 18.4%, attributed mostly to an increase of urinary urea N. Furthermore, findings from a meta-analysis on growing cattle offered a CP supplement indicated that up to 90% of incremental N intake that exceeds the requirement of rumen microbial activity is partitioned into urine (Huuskonen et al., 2014). This was in agreement with results from the present study, in which 38 g/d of the 42 g/d incremental CP was excreted as urinary N, a figure close to the predicted value of 37.8 g/d. Our results showed that feeding low-protein concentrates (14.1% CP) may serve as a mitigation strategy for reducing urine N output for cows consuming fresh-cut grass and

concentrate diets, reducing environmental footprint (N_2O emissions, nitrate and ammonia pollution) from pasture-based systems. Reducing the CP concentration of ruminant diets has been recommended as the most effective method of reducing N_2O emissions from dairy farms; it was estimated to cause a 7-fold improvement in mitigation efficiency compared with alleviating N_2O emissions through manure storage and management (Marini and Van Amburgh, 2005).

Our work showed that feeding low CP concentrates in a fresh-cut grass-based diet could shift N excretion from urine to feces when expressed as a proportion of manure N output. Regarding environmental concerns associated with grazing livestock, the shift of N excretion is desirable because N in feces is less volatile than in urine and may be converted to ammonia and N_2O at a slower rate (Van der Weerden et al., 2011). This is because fecal N is for the most part organically bound, composed mainly of microbial and endogenous N with some undigested feed N (Ellis et al., 2011), which must first undergo mineralization; urinary N is primarily in the form of urea, which is rapidly hydrolyzed to ammonium (Beukes et al., 2011).

Pure and crossbred Holstein cows showed similar NUE, in line with Huhtanen et al. (2008), who suggested dietary components may have a greater influence on milk protein N efficiency than level of production, although it also plays a role.

Development of Regression Equations Estimating Urine N Excretion for Grazing Dairy Cows

Previous work has shown that MUN concentration and urine N output are positively associated with dietary CP level, most likely a result of increased BUN (Jonker and Kohn, 2001; Huhtanen et al., 2015); therefore, MUN has been suggested as a noninvasive indicator for urine N excretion (Jonker and Kohn, 2001). Concentration of MUN is highly related to dietary CP content, and measurement is common practice in the dairy industry. However, differences exist between regression equations presented in the current study (equation [2], Table 6) and in previous studies, in which prediction equations were developed with animals fed diets based on conserved forage (Nousiainen et al., 2004; Spek et al., 2013). These differences may be a result of a combination of factors, such as animal diets, stage of lactation, genetic merit, and analytical techniques. Regression equations developed in the current study showed that urinary N output is positively related to MUN output and dietary CP content, which can be used as readily available predictors in practice. Positive relations between urine N output and MUN concentration have been found previously and explained by

the small neutral nature of a urea molecule, allowing MUN to equilibrate with BUN via diffusion into and out of the mammary gland (Jonker and Kohn, 2001). Spek et al. (2013) also found that urine N outputs' best sole predictors were feed CP content and MUN content. The fact that addition of dietary CP content to MUN content as predictors of urine N output only slightly improved R^2 in the present study implies that in practice the use of dietary CP content can be omitted without substantial compromise of prediction accuracy, when only routinely collected farm-level MUN content data are available. This allows for readily available, relatively reliable and inexpensive estimations of urine N excretions in pasture-based systems. The model we developed predicted urine N excretion to increase by 14.2 g/d with an increase of 1 g in MUN secreted, within the range of MUN values measured in the current study.

Mitigating NUE in dairy cattle requires reducing urinary N output without compromising—and preferably increasing—milk protein N yields. Because the majority of milk N is presented as protein, and protein yields depend on energy supplies, optimizing dietary energy supply but offering minimal levels of dietary CP without reducing productivity and milk solid concentrations would show high potential for mitigating N outputs in pasture-based systems.

CONCLUSIONS

The current results suggest urine N excretion from grazing lactating dairy animals can be alleviated by offering a concentrate with a CP level of 14.1% DM when good-quality perennial ryegrass is consumed. This practice can also reduce urine N as a proportion of total N excretion, which is considered environmentally desirable, because it decreases the volatilization of nitrogenous compounds, including N_2O emissions. Feeding the low CP concentrate did not affect voluntary grass intake, total intake, or production traits, implying that the proposed mitigation strategy should not compromise the economic performance of the dairy farm, although sustainability of production would have to be confirmed in a long-term study. The linear and multiple relationships developed in the current study may assist in the estimation of urine N output from animals fed fresh grass and concentrate diets, using readily available data at a commercial level, such as MUN data in conjunction with feed chemical composition or not.

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