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INTERPRETIVE SUMMARY

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

Manure nitrogen from dairy herds is a major source of pollution of air and ground water. The aim of this study was to reduce nitrogen output in dairy cows' manure, while sustaining milk production, by feeding low protein concentrates. When good quality grass was fed, reducing concentrates crude protein level from 18.1 to 14.1% (dry matter basis) had no adverse effect on milk production, but decreased urine nitrogen outputs. This may mitigate nitrogen pollution from grazing dairy herds, without comprising production efficiency. Linear and multiple relationships estimating urinary nitrogen, to be used at farm, research and policy-making levels, were produced.

RUNNING HEAD: URINARY NITROGEN ALLEVIATION

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

There are increased concerns regarding N pollution of air and ground water from grazing cattle. Although a number of studies have investigated mitigation strategies for N output from dairy cows fed conserved forages and concentrates, similar research on fresh-cut grass in addition to production parameters is limited. Therefore the current study, using 3 dietary treatments and incorporating 2 genotypes, was designed to evaluate the effects of concentrate crude protein (**CP**) level on animal production and N utilization efficiency (**NUE**) of lactating dairy cows. Twelve multiparous cows (6 Holstein and 6 Holstein × Swedish Red) were used in a change-over 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) fed at 32.8% DM intake in combination with good quality zero-grazed perennial ryegrass (18.2% CP, DM basis). Each period consisted of an adaption phase (18-d) housed as a single group, 1-d adaption in individual stalls and a 6-d measurement phase with feed intake and feces, urine and milk output recorded. There was no significant interaction between cow genotype and concentrate CP level on any animal performance or NUE parameters. Total DM intake, milk yield and composition and NUE were not affected by dietary treatment. However, increasing concentrate CP level increased (i) N intake by 42 g/d and excretion in urine and manure, by 38 and 40 g/d, respectively, and (ii) 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 consequently 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 sole predictor or in combination with dietary CP level. The present study indicated that when grazing cows are fed on 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 a successful method of reducing urinary N excretion of lactating dairy cattle on pasture-based systems, but further research is needed to investigate 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 ruminant, are a major source of environmental concern. With normal bovine feeding practises, a large percentage of dietary protein is inefficiently utilized leading to increased manure N outputs resulting in environmental, health (Butler, 1998) and economic implications. Excess N excretions from ruminants can be converted to many forms such as (i) ammonia, a major air pollutant, (ii) N_2O, a greenhouse gas, and (iii) 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). As controlling forage nutrient composition can prove difficult, 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 due to pasture often possessing a CP content in excess of or close proximity to 20% on DM basis (Kavanagh et al., 2003), a value considerably greater than that typically found in conserved forage. Hence, it is vital N partitioning is assessed in all commonly used farming practices to reduce pollution and maintain herd health in a cost-effective manner across the different dairy production systems. Previous studies have shown improved NUE in particular reduced urinary N excretion via reduced concentrate CP level (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 grass based diet is yet to be determined.

There is also evidence of 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 as a tool 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 animals. It has been speculated some of the variation may be explained by milk yield (MY) and BW (Huhtanen et al., 2015) or as a result of genetic variation in urea transporters located 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 which 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 Holstein breed (Heins et al., 2012). As Holstein and Swedish red represent important bovine breeds for MY and solids output, a comparison between Holstein and Holstein × Swedish red crossbreds would be suitable to examine the genetic and physiological effects on variation of N partitioning in dairy cattle.

Therefore, the objective of the present study was to (i) investigate the effects of animal genetics and varying concentrate CP content on production levels in combination with NUE and N partitioning parameters and (ii) 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 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 within each genotype were blocked into 3 groups of 2 cows, based on MY, BW and lactation stage, and were then randomly allocated to 3 dietary treatments. The mean MY, BW and DIM at the commencement of the trials 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: (i) an initial 18-d feed adaption phase where cows were housed as a single group with individual feed intake recorded, (ii) a 1-d adaption phase in individual stalls, and (iii) 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 and both contained the same feed ingredients and similar chemical composition (with the exception of CP content). Subsequently the MCP concentrate was then produced by mixing LCP and HCP in a 1:1 (w/w) ratio. The ingredient and chemical compositions 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) and half at afternoon milking (1500), while fresh-cut grass, harvested with a Haldrup 1500 from a single sward, was offered at 1000 each morning ad libitum. Herbage received primary cut during April 2014 and was subsequently harvested at regrowth intervals according to month (increasing from 22 to 30-d from June to September), generating grass of a similar quality to that under commercial management. Grass in the sward consisted of a three year re-seed of Aberstar, Aberzest and Alice varieties, sown in ratio of 8:5:1 respectively and paddocks had not been grazed since the end of the previous grazing season (November 2013). Post-harvesting fertilisation was implemented within 3-d at 35 kg N/ha. Temperature of fresh-cut grass was monitored throughout the study to minimise 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

Bodyweight was recorded before and after the digestibility unit phase. Daily herbage intakes and refusals 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**) contents 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 method in Jiao et al. (2013). A sub-sample of the bulked 3-d feces samples were 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, sulphuric acid solution (50% H_2SO_4) was added to the urine canisters prior to 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, Hilleröd, Denmark) for fat, protein and lactose. Contents of MUN were measured by the QuantiChrom urea assay kit (DIUR-500) after a de-proteination step (BioAssay Systems, Hayward, USA). 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 were performed as described previously by Cushnahan and Gordon (1995). For the analysis of grass, concentrate and milk concentrations of GE a Parr 6300 oxygen bomb calorimeter (Parr Instrument Company, Illinois, USA) 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 Fibretec fiber analyzer (Foss, Denmark).The NDF was assayed with a method using sodium sulphite and α-amylase, as described by Van Soest et al. (1991). Total starch content of concentrate was measured using 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 utilizing the Technicon Autoanalyzer (Technicon Corp., New York, NY; Thomas, 1977).

Statistical Analysis

Energy-corrected MY (**ECMY**) was calculated as milk energy output (MY multiplied by measured milk energy concentration) divided by milk energy content in one 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 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 a F Statistic against a F-distribution. Residuals showed no deviation from normality. The differences between treatments, genotypes and interactions were assessed and declared as non-significant, 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 concentrations 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 for the prediction of 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 which describes the squared correlation of the response and the fitted values, to represent the amount of variability explained was also generated.

RESULTS

The effect of the main factors was significant on a number of feed/nutrient intake, production and NUE parameters investigated, but there was no significant interaction between cow genotype and dietary treatment. Hence focus in the results and discussion sections will primarily be on main treatment effects.

Diet Composition

The chemical composition of individual dietary components is given in Table 2. Grass NDF and ADF contents both decreased and WSC contents increased from July through to September; but no seasonal variation was observed for ash, CP and GE contents of grass. The perennial ryegrass offered during the present experiment contained on 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. 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 level had no significant effect on voluntary feed intake and milk production and composition. In contrast, cow genotype had significant effect on feed intake, animal characteristics and milk production and composition parameters. We found 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 contents (+3.4%) but lower milk protein contents (-10.5%).

Nitrogen Partitioning and Utilization

The effects of concentrate CP contents and cow genotype on N intake, outputs and utilization variables are displayed in Table 4. We observed intakes of total and digestible N increased linearly with increasing concentrate CP content. Cows fed HCP diet consumed 42 g/d (total N) and 37 g/d (digestible N) more than those fed LCP diets. Feeding LCP concentrates significantly and linearly reduced urine N excretion compared to feeding HCP concentrates (-

38 g/d). We found excretion of manure N increased linearly with increasing concentrate CP content (+ 40 g/d for cows offered the HCP diet in comparison to those fed the LCP diet). Dietary treatment exerted no significant effect on N outputs in feces and milk, retained N and a number of NUE parameters (proportion of N intake excreted in feces, urine, manure, milk, and the ratio of retained to digested N). On the contrary, we observed a shift in N excretion from urine to feces when expressed relative to manure N, with proportion of urine N significantly decreased and proportion of feces N significantly increased when the LCP diet was fed, in comparison to the HCP.

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

MUN Output

Milk urea N output values are shown in Table 5. We observed MUN output linearly increased with increasing concentrate CP content, resulting in MUN values of cows fed HCP diet being on average 1.08 g/d higher than cows offered LCP diet. We also found MUN concentrations declined linearly with decreasing concentrate CP content (-1.6 and -3.6 mg/dL for cows offered MCP and LCP in comparison to 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, concentrations or proportion to total N or digestible N intakes was not significant.

Estimation of MUN and Urine Nitrogen Output

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

MUN output and MUN concentrations respectively, and (ii) MUN and dietary CP content for the prediction 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 Eq. 3 in Table 6.

DISCUSSION

The manipulation of concentrate CP concentration is commonly used to optimize rumen microbial activity and consequently 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 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 and reductions in carbohydrate content occur during ensiling. In addition daily deviations in pasture CP content are more pronounced in comparison to conserved forage which may also affect the ruminal protein-energy balance. The present study was thus 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 utilized in the present study would be considered typical for good quality ryegrass (Ministry of Agriculture, Fisheries and Food, 1992). Water-soluble carbohydrates content of fresh-cut grass increased between July and September, which is possibly due to longer grass regrowth intervals towards the end of the grazing season (Owens et al., 2008). Throughout the present experiment, good quality ryegrass averaging 18.2% CP, 461 g/kg NDF and 162 g/kg

WSC, was offered. Consequently animals consumed higher than the 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 two factors in combination may reduce 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 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 may sustain MY 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 MY of 2.9 kg/d when compared to feeding a 19% CP concentrate (Whelan et al., 2012), while 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 as low as 14% might be fed to dairy cows without negative implications on milk production (Sinclair et al., 2014). There is a range of diet and animal factors which could influence the effect of concentrate CP levels on milk production of grazing cows, such as milk production potential, stage of lactation and forage quality (de Oliveira et al., 2010; Moran, 2005). In the present study, 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.

Nitrogen Partitioning and Utilization

In the present study we observed that increasing concentrate CP levels in a predominantly fresh ryegrass diet supplemented with concentrate increased total intakes 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 is similar to those observed in previous literature (Ruiz et al., 2001; Lee et al., 2009; Kebreab et al., 2010). In the present study, the non-significant effect of concentrate CP concentration on feces N excretion was partially due to similar DMI, an influential factor in fecal N output, between treatments. It 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 on the HCP diet. In comparison to the LCP diet, the additional N intake in the HCP diet (42 g/d) was almost entirely excreted in urine (38 g/d), which displays 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% which was attributed mostly to an increase of urinary urea N. Furthermore, findings from a meta-analysis on growing cattle offered CP supplement indicates that up to 90% of incremental N intake, which exceeds the requirement of rumen microbial activity, is partitioned into urine (Huuskonen et al., 2014). This is in agreement with results from the present study, in which 38 (g/d) out of the 42 (g/d) incremental CP was excreted as urinary N, a figure which is 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 to reduce urine N output for cows consuming fresh-cut grass and concentrate diets, thus reducing environmental footprint (N_2O emissions, nitrate and ammonia pollution) from pasture-based systems. Reducing CP concentration of ruminant diets has been recommended to be the most effective method to reduce N_2O emissions from dairy farms; it was estimated to cause a 7-fold improvement on 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 considered 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 due to fecal N being for the most part organically bound N composing mainly of microbial and endogenous N with some undigested feed N (Ellis et al., 2011), which must first undergo mineralization whereas 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, thus being 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, though it too plays a role.

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

Previous work has shown MUN concentration and urine N output are positively associated with dietary CP level, which is most likely a result of increased BUN (Jonker and Kohn, 2001; Huhtanen et al., 2015); therefore MUN has been suggested as a non-invasive indicator for urine N excretion (Jonker and Kohn, 2001). MUN concentration 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 (Eq. 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 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 on the prediction accuracy, when only routinely collected at farm-level MUN content data is available. This allows for readily available, relatively reliable and non-expensive estimations of urine N excretions in pasture-based systems. The model we developed predicts 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 but without compromising, and preferably increasing, milk protein N yields. As the majority of milk N is presented as protein and protein yields are dependent on energy supplies, optimising dietary energy supply while offering minimal levels of dietary CP, without reducing productivity and milk solid concentrations, would show high potential to mitigate N outputs in pasture-based systems.

CONCLUSION

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 as it decreases 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 economic performance of the dairy farm, although sustainability of production would have to be confirmed on 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 commercial level, such as MUN data either in conjunction with feed chemical composition or not.

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FIGURE CAPTIONS

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 Eq. 3 in Table 6.

TABLES

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

	LCP[1]	HCP[1]
Corn	246	220
Wheat feed	140	135
Corn gluten	140	135
Soya hulls	140	135
Palm kernel exp.	110	110
Sugar beet pulp	45	0
Sunflower kernel	66	60
Soyabean 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

[1]LCP = low CP concentrate (14.1%, DM basis); HCP = high CP concentrate (18.1%, DM basis).
[2]Trace 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 vitamin A, 2,000 IU / kg vitamin D3.

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

	Grass			Concentrate	
	July	August	September	LCP[1]	HCP[1]
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 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		

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

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

	Concentrate CP level				P-value[1]		Cow genotype			
	Low	Medium	High	SEM	L	Q	Holstein	Crossbred[2]	SEM	P-value
Animal characteristics										
BCS	2.37	2.30	2.34	0.038	0.46	0.22	2.29	2.39	0.044	0.12
Bodyweight, kg	579	582	571	15.2	0.32	0.43	583	573	20.8	0.74
Feed intake, kg DM/d										
Grass intake	13.8	14.1	14.1	0.37	0.30	0.24	14.4	13.5	0.32	0.009
Concentrate intake	7.0	7.0	6.9	0.16	0.61	0.40	7.0	6.9	0.16	0.30
Total DM intake	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
Energy corrected milk 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

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

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

	Concentrate CP level				P-value[1]		Cow genotype			
	Low	Medium	High	SEM	L	Q	Holstein	Crossbred[2]	SEM	P-value
N intake/output, g/d										
Total dietary N intake	543	572	585	16.6	<0.001	0.17	579	554	12.6	0.039
Digestible N intake	358	382	395	13.7	<0.001	0.038	388	369	12.7	0.044
Feces N	187	187	188	6.4	0.86	0.81	190	185	6.0	0.55
Urine N	193	208	231	10.8	0.004	0.63	220	202	10.6	0.25
Manure N	380	394	420	12.8	0.017	0.65	409	387	12.5	0.24
Milk total N	149	154	156	5.5	0.41	0.89	157	149	6.2	0.42
Milk protein N	144	149	150	5.2	0.54	0.91	151	145	5.6	0.074
Retained N	15.4	22.3	7.9	15.54	0.61	0.17	14	17	15.6	0.86
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	0.87
Urine N /N intake	0.356	0.363	0.402	0.0210	0.054	0.46	0.380	0.367	0.0214	0.65
Manure N /N intake	0.701	0.694	0.727	0.0253	0.29	0.35	0.711	0.703	0.0270	0.81
Milk total N /N intake	0.274	0.271	0.270	0.0085	0.63	0.88	0.272	0.271	0.0076	0.88
Milk protein N /N intake	0.265	0.262	0.260	0.0080	0.51	0.90	0.265	0.262	0.0074	0.78
Retained N /N intake	0.024	0.036	0.004	0.0288	0.43	0.12	0.017	0.026	0.0289	0.80
Feces N /Manure N	0.497	0.478	0.452	0.0157	0.007	0.77	0.469	0.481	0.0158	0.54
Urine N /Manure N	0.503	0.522	0.548	0.0157	0.007	0.77	0.531	0.519	0.0158	0.54

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

	Concentrate CP level				P-value[1]		Cow genotype			
	Low	Medium	High	SEM	L	Q	Holstein	Crossbred[2]	SEM	P-value
MUN, g/d	4.85	5.35	5.93	0.476	0.016	0.86	5.82	4.89	0.473	0.13
MUN content, mg/dL	18.9	20.9	22.5	1.23	<0.001	0.75	20.7	20.7	1.26	0.96
MUN /N intake	0.0090	0.0094	0.0103	0.00087	0.093	0.41	0.0101	0.0090	0.00087	0.20
MUN /Digestible N intake	0.0141	0.0141	0.0157	0.00155	0.29	0.23	0.0155	0.0137	0.00156	0.26

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

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

Equation no.		Equations[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 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

R^2 = pseudo correlation coefficient.

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

Figures

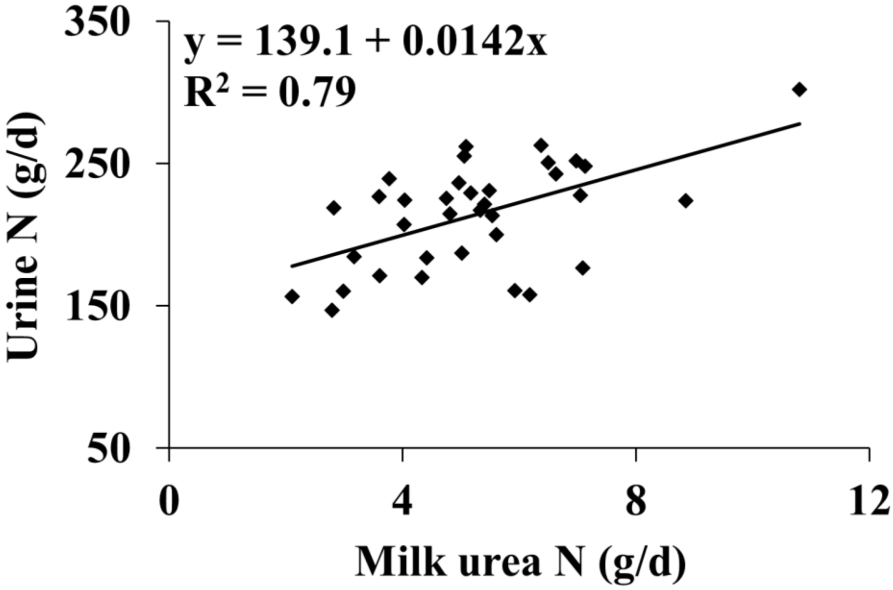

Figure 1.