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Effects of dietary crude protein concentration on animal performance and nitrogen utilisation efficiency at different stages of lactation in Holstein-Friesian dairy cows

C.T. Yang, C.P. Ferris, T. Yan *

Agri-Food and Biosciences Institute, Hillsborough, Co. Down, United Kingdom

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ABSTRACT

Nitrogen (**N**) excretion from livestock production systems is of significant environmental concern; however, few studies have investigated the effect of dietary CP concentration on N utilisation efficiency at different stages of lactation, and the interaction between dietary CP levels and stages of lactation on N utilisation. Holstein-Friesian dairy cows (12 primiparous and 12 multiparous) used in the present study were selected from a larger group of cows involved in a whole-lactation study designed to examine the effect of dietary CP concentration on milk production and N excretion rates at different stages of lactation. The total diet CP concentrations evaluated were 114 (low CP), 144 (medium CP) and 173 (high CP) g/kg DM, with diets containing (g/kg DM) 550 concentrates, 270 grass silage and 180 maize silage. During early (70–80 days), mid- (150–160 days) and late (230–240 days) lactation, the same 24 animals were transferred from the main cow house to metabolism units for measurements of feed intake, milk production and faeces and urine outputs. Diet had no effect on BW, body condition score, or milk fat, protein or lactose concentration, but DM intake, milk yield and digestibilities of DM, energy and N increased with increasing diet CP concentration. The effect of diet on milk yield was largely due to differences between the low and medium CP diets. Increasing dietary CP concentration significantly increased urine N/N intake and urine N/manure N, and decreased faecal N/N intake, milk N/N intake and manure N/N intake. Although increasing dietary CP level significantly increased urine N/milk yield and manure N/milk yield, differences in these two variables between low and medium CP diets were not significant. There was no significant interaction between CP level and stage of lactation on any N utilisation variable, indicating that the effects of CP concentration on these variables were similar between stages of lactation. These results demonstrated that a decrease in dietary CP concentration from high (173 g/kg DM) to medium level (144 g/kg DM) may be appropriate for Holstein-Friesian dairy cow to maintain milk production efficiency, whilst reducing both urine N and manure N as a proportion of N intake or milk production.

Implications

Nitrogen excretion from livestock production systems is of significant environmental concern at present. Large quantities of nitrogen are brought onto dairy farms in feedstuff, and much of this nitrogen is lost to the environment rather than being incorporated into milk or animal tissue. Feeding diets containing marginally lower nitrogen levels than commonly practised or recommended by feed rationing systems could not only maintain milk production efficiency but also increase nitrogen utilisation, decrease feed costs and reduce nitrogen losses to the environment. Findings from this study can help producers make effective decisions on maintaining production efficiency while reducing their environmental footprint.

Introduction

Nitrogen (**N**) excretion from livestock production systems is of significant environmental concern at present and contributes to atmospheric emissions of ammonia and nitrous oxide, and can lead to nitrate contamination of surface and groundwater (Barros et al., 2017; Hoekstra et al., 2020). Large quantities of N are brought onto dairy farms, primarily in feed and fertiliser, and much of this N is lost to the environment rather than being incorporated into milk, animal tissue and crops (Ding et al., 2020). The overall efficiency

* Corresponding author.
E-mail address: tianhai.yan@afbini.gov.uk (T. Yan).

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of utilisation of dietary N in European dairy farming was estimated to be less than 25%, and is decreasing (Chen et al., 2020). Previous studies have shown that many dietary and animal factors influence manure N output in lactating dairy cows (Kidane et al., 2018; Nichols et al., 2019). However, dietary N concentration and N intake are the two most important factors due to their strong positive relationships with manure N output in dairy cows (Barros et al., 2017). Feeding diets containing marginally lower CP levels than commonly practised (Aguerre et al., 2016; Husnain and Santos, 2019), or recommended by feed rationing systems (Agricultural and Food Research Council (AFRC), 1993; National Research Council (NRC), 2001), has been shown to have only a small impact on milk production, but could significantly increase dietary N utilisation efficiency, decrease feed costs and reduce N losses to the environment (Olmos Colmenero and Broderick, 2006; Hristov et al., 2011). For example, decreasing dietary CP concentration from 181 to 141 g/kg DM (Hynes et al., 2016) or from 166 to 153 g/kg DM (Aguerre et al., 2016) did not influence DM intake or milk yield, but increased N utilisation efficiency of dairy cows. Similarly, Barros et al. (2017) found that a reduction in dietary CP level from 162 g/kg DM to 144 g/kg DM had no effect on milk yield, whereas a further reduction to 131 or 118 g/kg DM resulted in a rapid fall in milk yield (after 4 and 1 weeks of offering the experimental diets, respectively). However, these data (Aguerre et al., 2016; Hynes et al., 2016; Barros et al., 2017) were obtained from short-term feeding studies and should be interpreted with caution when extrapolated to the whole-lactation period. Few studies have investigated the effect of dietary CP concentration on N utilisation efficiency at different stages of lactation, and the interaction between dietary CP levels and stages of lactation on N utilisation. Therefore, the objectives of the present study were to evaluate the effect of dietary CP concentration on milk production, nutrient digestibility and N utilisation efficiency of lactating dairy cows during early, mid- and late lactation, and the interactions between dietary CP concentration and stage of lactation on N utilisation efficiency.

Material and methods

Experimental design, animal and diets

The 24 cows used in the present experiment comprised a subgroup of the cows involved in a whole-lactation study, as described in full in Supplementary Material S1. Briefly, the whole lactation study used 90 autumn-calving Holstein-Friesian dairy cows (45 primiparous and 45 multiparous) which were randomly allocated to total mixed rations (**TMRs**) containing 1 of 3 dietary CP concentrations (114, 144 or 173 g CP/kg DM: Low CP, Medium CP, and High CP, respectively) from calving until day 150 of lactation. Although half of the animals on each treatment changed to a diet containing a different protein level at day 151 of lactation, the 24 cows used on the current study remained on their original diet CP level throughout the entire lactation. Eight cows from each CP treatment (four primiparous and four multiparous) were selected prior to the first measurement period, with cows across treatments balanced for parity and calving date. During early (70–80 days), mid- (150–160 days) and late (230–240 days) lactation, these cows were transferred to metabolism units for total diet digestibility and nutrient utilisation measurements. Diets were offered in the form of a TMR and contained (g/kg DM) 270 grass silage, 180 maize silage and 550 concentrates (Table 1). Three target diet CP concentrations were achieved by manipulating the proportions of two concentrate meals which differed in CP levels. The two concentrates contained the same level of metabolisable energy (13.1 MJ/kg DM), but different concentrations of CP (229 vs 117 g/kg DM).

Table 1
Metabolisable energy (ME) and protein concentrations of the mixed diets offered for dairy cows.

Item	Low CP	Medium CP	High CP
ME[1] (MJ/kg DM)	12.4	12.4	12.4
CP (g/kg DM)	114	144	173
Rumen degradable protein (g/kg DM)	78.1	95.4	111.1
Diet undegradable protein (g/kg DM)	35.9	48.6	61.9

[1] Dietary ME concentration was calculated using measured gross energy intake and faeces and urine energy outputs, and estimated methane energy output (calculated as 6.8% of gross energy intake (Yan et al., 2010)).

These variables were calculated using the UK Feed into Milk programme (Feed into Milk, 2004). The cows were offered fresh food *ad libitum* between 1000 and 1100 h daily, had free access to water, and were milked twice daily starting at 0500 and 1630 h, respectively.

Digestibility study and sample measurements

Throughout the study, from calving to 305 day of lactation, total feed intake was recorded daily. During early (70–80 days), mid- (150–160 days) and late (230–240 days) lactation, the animals from each treatment selected for the present digestibility measurements were transferred to metabolism units and housed in individual stalls for 8 days, with feed intake measured daily and outputs of faeces and urine measured during the final 6 days (measurement period). During the measurement period of the present digestibility study, the grass silage and maize silage were sampled daily for determination of fermentation variables (pH, volatile fatty acids, ammonia N, lactic acid, ethanol and propanol concentrations), N and gross energy concentrations, and oven DM concentration (60 °C for 48 h). These oven-dried silage samples were then bulked over the measurement period for the determination of ash, NDF and ADF concentrations. Samples of concentrate were taken daily and bulked over the 6 days for determination of DM (85 °C for 24 h), ash, gross energy, N, NDF and ADF concentrations. Faeces and urine outputs were recorded daily, and daily samples were taken as a proportion (5%) of total excretion of faeces (by weight) and urine (by volume). The 6-day samples of faeces and urine for each cow were separately mixed and a representative sample was taken for analysis as follows: fresh faeces samples for oven DM (85 °C for 48 h), gross energy and N concentrations, and urine samples for gross energy and N concentrations. The methods adopted for analysis of silage, concentrate, faeces and urine samples were as described by Cushnahan and Gordon (1995). Briefly, silage DM concentrations were calculated from oven DM concentrations (60 °C for 48 h) corrected for the loss of volatile fatty acids, lactic acid, alcohol and ammonia (Porter and Murray, 2001). Silage ammonia concentration was assayed by bringing the alkalinity of the aqueous extract to pH 10 and using an Orion 95-12-00 ammonia sensing gas electrode. The volatile fatty acids, lactic acid, ethanol and propanol concentrations in silage samples were analysed by GLC using a Perkin-Elmer gas chromatograph having a column packed with matrix 80/120 Carbopack B-DA and 4% Carbowax 20 M. Ash concentration in silage, concentrate and faeces samples was determined by incineration in a muffle furnace (Vecstar, Derbyshire, UK) at 550 °C for approximately 10 h (Association of Official Analytical Chemists (AOAC), 1990). The N concentrations of fresh silage, concentrate, fresh faeces and urine samples were determined using a Tecator Kjeldahl Auto 1030 Analyzer (Foss Tecator AB, Höganäs, Sweden). Gross energy concentrations in silage, concentrate, faeces and urine samples were measured in an isoperibol bomb calorimeter (Parr Instruments Co., Moline, IL). The NDF and ADF concentrations in silage, concentrate and faecal samples were determined sequentially using a Fibertec fibera analyzer (Foss Electric, Hillerød, Denmark). The NDF was assayed using sodium sulfite and α-amylase, as described by Van Soest et al. (1991).

During the measurement period, milk yield was recorded daily and milk samples were taken daily during the morning and afternoon milking and analysed for fat, N, lactose and energy concentrations. Fat and lactose concentrations were analysed by the method of Ling (1963) using a Milkoscan Model 605 (Foss Electric, DK-3400, Hillorod, Denmark), and N and energy concentrations were determined as described above.

Cow BW and body condition score were determined before the morning feeding on the first and last day in digestibility measurements. Body condition of each cow was determined using the method described by Edmonson et al. (1989), with five categories from one (very thin) to five (very fat).

Statistical analysis

A repeated measure approach using the residual maximum likelihood procedure was used to analyse the data set. The model fitted fixed effects for dietary protein treatment and stage of lactation for each parameter. The model included all two-level interactions between these variables. Orthogonal contrasts were calculated for linear and quadratic effects of diet treatments. The statistical program used in the present study was Genstat 6.1 (sixth edition, Lawes Agricultural Trust, Rothamsted, UK) with a probability level of $P = 0.05$ for significance in treatments and interactions.

Results

Feed intake and milk yield

There was no significant interaction between dietary CP level and stage of lactation for any feed intake or milk production variable (Table 2). However, increasing dietary CP concentration linearly ($P < 0.001$) increased total DMI, but had no significant effect on BW and body condition score. Increasing dietary CP concentration from Low to Medium levels significantly increased milk yield, energy-corrected milk (**ECM**) yield, component yields of fat, protein and lactose, and milk energy output, while further increasing dietary CP concentration from Medium to High levels had no significant effects on these variations. However, dietary CP concentration had no effects on milk fat, protein, lactose and energy concentrations. Cows in late lactation had a significantly higher BW and body condition score, but a significantly lower milk yield, milk component yield (fat, protein and lactose) and milk energy output, when compared with those in early and mid-lactation.

Energy and nutrient digestibility

There was no significant interaction between dietary CP level and stage of lactation for any digestibility variable (Table 3). Increasing dietary CP concentration resulted in a linear increase in DM, energy and N digestibility, although differences in DM and energy digestibility between Low and Medium CP levels were not significant. In addition, cows in early lactation had a significantly lower energy digestibility when compared with those in mid- and late lactation.

Nitrogen utilisation efficiency

There was no significant interaction between dietary CP level and stage of lactation for any N utilisation efficiency variable (Table 4). Increasing dietary CP concentration linearly ($P < 0.001$) increased N intake, N outputs in faeces and urine, and N incorporated into milk and body tissue, while milk N showed a quadratic ($P = 0.045$) response to dietary CP level. Increasing dietary CP concentration also linearly increased ($P < 0.001$) urine N/N intake, urine N/milk yield, manure N/milk yield, urine N/ECM yield, manure N/ECM yield and urine N/manure N, while linearly decreasing ($P \leq 0.002$) faecal N/N intake, milk N/N intake and manure N/N intake. However, there was no significant difference in urine N/milk yield, manure N/milk yield, manure N/ECM yield, and milk N/N intake between Low and Medium CP diets. Cows in late lactation excreted significantly more N in urine, and had significantly higher ratios of urine N/N intake, manure N/N intake, urine N/manure N, and N excretion as a proportion of milk yield, and a significantly lower daily milk N output and milk N/N intake, when compared with those in early and mid-lactation.

Table 2
Effects of dietary CP concentration on BW, feed intake, milk yield and milk composition of dairy cows.

Item	Dietary CP level			SE	*P*-value[1]		Stage of lactation			SE	*P*-value
	Low	Medium	High		L	Q	Early	Mid	Late		
BW and feed intake											
BW (kg)	554	537	536	13.7	0.359	0.618	508[b]	535[b]	584[a]	7.9	0.001
Body condition score	2.52	2.43	2.44	0.055	0.286	0.441	2.34[b]	2.43[b]	2.62[a]	0.032	0.003
Total DM intake (kg/d)	16.5[b]	18.4[a]	19.5[a]	0.43	<0.001	0.450	18.4	18.4	17.6	0.25	0.280
Milk yield (kg/d)	20.6[b]	26.1[a]	28.3[a]	0.93	<0.001	0.160	27.9[a]	26.2[a]	20.9[b]	0.54	<0.001
ECM yield (kg/d)	20.7[b]	25.7[a]	27.8[a]	0.78	<0.001	0.130	26.8[a]	26.1[a]	21.3[b]	0.45	<0.001
Milk composition (g/kg)											
Fat	41.9	40.4	39.7	1.38	0.261	0.832	38.5	40.9	42.7	0.80	0.104
Protein	34.0	35.3	35.9	0.94	0.169	0.787	31.5[c]	38.6[a]	35.1[b]	0.54	<0.001
Lactose	47.2	45.9	46.3	0.87	0.463	0.413	48.9[a]	43.1[b]	47.5[a]	0.503	<0.001
Energy (MJ/kg)	3.14	3.09	3.08	0.058	0.436	0.798	3.00	3.12	3.19	0.033	0.074
Milk component yield (kg/d)											
Fat	0.85[b]	1.03[a]	1.10[a]	0.036	<0.001	0.172	1.05[a]	1.05[a]	0.88[b]	0.021	0.001
Protein	0.69[b]	0.91[a]	1.02[a]	0.038	<0.001	0.271	0.88[b]	1.01[a]	0.73[c]	0.022	<0.001
Lactose	0.98[b]	1.20[a]	1.30[a]	0.048	<0.001	0.307	1.36[a]	1.12[b]	0.99[b]	0.027	<0.001
Milk energy output (MJ/d)	64.0[b]	79.7[a]	86.0[a]	2.42	<0.001	0.122	82.9[a]	80.8[a]	66.0[b]	1.40	<0.001

Abbreviation: ECM = energy-corrected milk.
[a–c] Means within a row for 'dietary CP level' or 'stage of lactation', with no common superscripts differ ($P < 0.05$).
[1] Probability of a linear (L) and quadratic (Q) effect of dietary CP level. The interaction of dietary CP level and stage of lactation was not significant ($P > 0.05$) for all response variables.

Table 3
Effects of dietary CP concentration on total ration digestibility variables (%) of dairy cows.

Item	Dietary CP level			SE	*P*-value[1]		Stage of lactation			SE	*P*-value
	Low	Medium	High		L	Q	Early	Mid	Late		
DM digestibility	72.4^{b}	73.0^{b}	74.7^{a}	0.50	0.001	0.318	73.0	74.0	73.2	0.30	0.296
Energy digestibility	71.7^{b}	72.4^{b}	74.2^{a}	0.50	<0.001	0.323	71.5^{b}	73.6^{a}	73.3^{a}	0.30	0.003
Nitrogen digestibility	57.5^{c}	63.7^{b}	69.2^{a}	1.00	<0.001	0.756	62.6	62.8	65.0	0.60	0.156

[a–c] Means within a row for 'dietary CP level' or 'stage of lactation', with no common superscripts differ ($P < 0.05$).
[1] Probability of a linear (L) and quadratic (Q) effect of dietary CP level. The interaction of dietary CP level and stage of lactation was not significant ($P > 0.05$) for all response variables.

Table 4
Effects of dietary CP concentration on nitrogen (N) utilisation efficiency of dairy cows.

	Dietary CP level			SE	*P*-value[1]		Stage of lactation			SE	*P*-value
	Low	Medium	High		L	Q	Early	Mid	Late		
N intake and output (g/d)											
N intake	322^{c}	445^{b}	562^{a}	10.6	<0.001	0.824	450	442	436	6.1	0.678
Faecal N	135^{b}	162^{a}	173^{a}	5.1	<0.001	0.204	163	158	149	2.9	0.158
Urine N	92^{c}	138^{b}	207^{a}	5.6	<0.001	0.092	140^{b}	139^{b}	157^{a}	3.2	0.040
Milk N	100^{b}	132^{a}	144^{a}	4.3	<0.001	0.045	133^{a}	133^{a}	110^{b}	2.5	<0.001
N balance	−4.9^{c}	12.8^{b}	38.3^{a}	1.88	<0.001	0.097	13.3^{b}	12.8^{b}	20.0^{a}	1.09	0.014
N partition (g/g)											
Faecal N/N intake	0.425^{a}	0.363^{b}	0.308^{c}	0.0097	<0.001	0.756	0.374	0.372	0.350	0.0060	0.156
Urine N/N intake	0.282^{c}	0.311^{b}	0.368^{a}	0.0082	<0.001	0.171	0.303^{b}	0.304^{b}	0.353^{a}	0.0050	<0.001
Milk N/N intake	0.310^{a}	0.297^{a}	0.256^{b}	0.0068	<0.001	0.113	0.301^{a}	0.304^{a}	0.258^{b}	0.0040	<0.001
Manure N/N intake	0.707^{a}	0.674^{b}	0.676^{b}	0.0065	0.002	0.033	0.678^{b}	0.675^{b}	0.703^{a}	0.0040	0.007
N balance/N intake	−0.017^{c}	0.030^{b}	0.068^{a}	0.0043	<0.001	0.462	0.021^{b}	0.021^{b}	0.040^{a}	0.0030	0.005
Urine N/manure N	0.399^{c}	0.462^{b}	0.543^{a}	0.0115	<0.001	0.526	0.448^{b}	0.453^{b}	0.504^{a}	0.0070	0.002
N excretion as a proportion of milk yield (g/kg)											
Faecal N/milk yield	6.75	6.45	6.37	0.439	0.391	0.772	5.97^{b}	6.26^{b}	7.33^{a}	0.179	0.008
Urine N/milk yield	4.64^{b}	5.54^{b}	7.72^{a}	0.317	<0.001	0.103	4.97^{b}	5.28^{b}	7.65^{a}	0.183	<0.001
Manure N/milk yield	11.4^{b}	12.0^{b}	14.1^{a}	0.533	0.001	0.256	10.9^{b}	11.5^{b}	15.0^{a}	0.308	<0.001
Faecal N/ECM yield	6.64	6.41	6.37	0.236	0.421	0.747	6.15^{b}	6.17^{b}	7.10^{a}	0.136	0.008
Urine N/ECM yield	4.57^{c}	5.54^{b}	7.72^{a}	0.275	<0.001	0.077	5.15^{b}	5.25^{b}	7.44^{a}	0.159	<0.001
Manure N/ECM yield	11.2^{b}	12.0^{b}	14.1^{a}	0.410	<0.001	0.172	11.3^{b}	11.4^{b}	14.5^{a}	0.237	<0.001

Abbreviation: ECM = energy-corrected milk.
[a–c] Means within a row for 'dietary CP level' or 'stage of lactation', with no common superscripts differ ($P < 0.05$).
[1] Probability of a linear (L) and quadratic (Q) effect of dietary CP level. The interaction of dietary CP level and stage of lactation was not significant ($P > 0.05$) for all response variables.

Discussion

Feed intake, digestibility and milk production

In the present study, increasing dietary CP concentration from Low (114 g/kg DM) to Medium (144 g/kg DM) levels significantly increased DM intake of lactating dairy cows, while a further increase in dietary CP concentration to High level (173 g/kg DM) had no effect on DM intake. The results were consistent with those obtained by Law et al. (2009a) in the main production study who suggested that increasing the amount of rumen degradable protein (**RDP**) intake with high CP diets should stimulate rumen microbial activity and consequently DM intake. Although increasing dietary CP levels from Medium to High significantly increased both DM (2.3%) and energy (2.5%) digestibilities, the increase was small, with the responses much smaller than the 26.1% increase in diet RDP intake in the present study. This suggested either the supply of fermentable energy was insufficient to match RDP intake in the High CP diet or that rumen bacterial growth was close to maximum in cows offered the Medium CP diet (Law et al., 2009a). In agreement with this, Cyriac et al. (2008) reported that cows offered diets containing 88 g/kg DM RDP (159 g CP/kg DM) were able to maintain DM intake and milk yield compared with cows offered high RDP diets (113 g/kg DM). The absence of a significant response in DM or energy digestibility between Low and Medium CP diets in the present study might suggest that the RDP supply with the Low CP diet did not place a considerable restriction on rumen microbial activity.

In the present study, a milk yield response to diet CP level was observed between the Low and Medium CP treatments, but not between the Medium and High treatments. However, although within the main study, Law et al. (2009a) reported a quadratic milk yield response when dietary CP concentration increased from 114 (Low CP) to 173 (High CP) g/kg DM, this result suggests that Holstein dairy cows from 151 to 305 days in milk could be offered diets containing CP levels as low as 144 g/kg DM (Medium CP) with no loss in milk production. Mutsvangwa et al. (2016) suggested that the ability of cows to maintain milk yields with low CP diets could be attributed in part to enhanced urea N recycling, thus increasing the supply of RDP for rumen microbial activity.

Nitrogen utilisation

In the present study, N intake increased significantly with increasing dietary CP level, with a resulting increase in N outputs in faeces and urine, and N incorporated into milk and body tissue. Increased N intake and outputs associated with higher CP diets have been documented in previous studies in dairy cows (Jiao et al., 2014; Stergiadis et al., 2015; Hynes et al., 2016) and beef cattle (Yan et al., 2007; Dong et al., 2014). In line with the reports of Niu et al. (2016) and Kidane et al. (2018), the present study observed a substantial portion (68–71%) of consumed N being excreted in urine and faeces. Furthermore, urine N values were more variable (92–207 g/d) than faecal N (135–173 g/d), which is similar to those observed in literature (Kebreab et al., 2010; Hynes et al., 2016). Compared with the Medium CP diet (144 g/kg DM), over 50% of the additional N intake (117 g/d) in the High CP diet (173 g/kg DM) was excreted in urine (69 g/d). Hynes et al. (2016) observed that feeding dairy cow diets with CP level of 183, rather than 169 g/kg DM, increased N intake (585 vs 543 g/d) by 7.7%, whereas a 19.7% increase in urine N output (231 vs 193 g/d) was observed. Furthermore, a meta-analysis with growing cattle found that up to 90% of incremental N intake that exceeded the requirement of rumen microbial activity was partitioned into urine (Huuskonen et al., 2014). These losses create a significant risk of pollution to the environment, and also represent a financial loss, as protein ingredients are often the most expensive component of the diet (Castillo et al., 2000; Kidane et al., 2018).

In the present study, dietary CP concentration and stage of lactation both had significant effects on N utilisation efficiency, when expressed as N outputs as a proportion of N intake or milk production. However, there was no significant interaction between CP level and stage of lactation on any variable of N utilisation, indicating the effects of CP concentration on these variables were independent, irrespective of stage of lactation.

In the present study, decreasing dietary CP level from High (173 g/kg DM) to Medium (144 g/kg DM) significantly reduced urine N/N intake and increased faecal N/N intake and milk N/N intake across the three periods, whereas there was no effect on manure N/N intake. This indicates that N digestibility is not a good indication of N utilisation efficiency, because the High CP diet had a significantly higher N digestibility than that of the Medium or Low CP diet. As discussed previously, the present High CP diet might over-supply RDP that exceeded the requirement of microbial activity in the rumen, consequently, the excess ammonia from the over-supplied degradable CP would be absorbed through the rumen wall into the bloodstream, and excreted from urine as urea (Bach et al., 2005; Mutsvangwa et al., 2016). However, a further decrease in dietary CP concentration from Medium (144 g/kg DM) to Low CP (114 g/kg DM) level in the present study had no significant effects on milk N/N intake or manure N/milk yield and increased manure N/N intake across the three periods. Therefore, these results indicate that reducing dietary CP concentration to Medium level (144 g/kg DM) may be appropriate for dairy cows to maintain milk production efficiency while reducing N loss in urine and manure.

Furthermore, reducing dietary CP levels from High to Low (173–114 g/kg DM) in the present study linearly decreased urine N/manure N. The shift in N excretion from urine to faeces with low CP diets is considered desirable and is of specific environmental importance. Ruminants excrete large amounts of N in pastoral soils which is a significant source of ammonia emissions, as urinary urea is rapidly hydrolysed to ammonium by the urease enzyme, whereas faecal ammonia production is generally low due to slow mineralisation rates of organic nitrogenous compounds (Schils et al., 2013; Waldrip et al., 2013).

Conclusions

The present study assessed the effects of dietary protein concentration on milk production and N utilisation efficiency of lactating dairy cows in early, mid- and late lactation. There was no significant interaction between dietary CP level and stage of lactation on N utilisation efficiency. Although feeding high CP diets could increase energy and N digestibility, reducing dietary CP concentration from 173 to 144 g/kg DM showed a marginal reduction in feed intake and milk yield, whilst decreased N excretion as a proportion of N intake or milk production. However, a further reduction of dietary CP level to 114 g/kg DM significantly decreased feed intake and milk production and increased manure N/N intake. Decreasing dietary CP concentration can also shift N excretion from urine to faeces, an environmentally desirable action that could decrease ammonia emissions. These results indicate that dietary CP concentrations as low as 144 g/kg DM may be appropriate for dairy cows to maintain milk production efficiency while reducing urine and manure N outputs. Further studies are required to validate the magnitude of effects of reduced dietary CP levels on milk production and N utilisation efficiency using larger groups of dairy cows.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100562.

Ethics approval

All procedures adopted in the present experiments were approved by the Ethical Review Committee of the Agri-Food and Biosciences Institute (Hillsborough, United Kingdom) under the Project Licence of 2587b and were in accordance with the UK Animal Scientific Procedures Act (1986).

Data and model availability statement

The data used in this study were deposited in the Agri-Food and Biosciences Institute (Hillsborough, UK) database. Data are available from the corresponding author on reasonable request.

Author ORCIDs

C.T.Y.: https://orcid.org/0000-0002-4354-2606.
C.P.F.: https://orcid.org/0000-0001-8623-5983.
T.Y.: https://orcid.org/0000-0002-1994-5202.

Author contributions

Conceptualisation: **C.P.F.** and **T.Y.** Methodology: **C.P.F.** and **T.Y.** Software: **T.Y.** Validation: **T.Y.** Formal analysis: **T.Y.** Investigation: **T.Y.** Data Curation: **T.Y.** and **C.T.Y.** Writing – Original Draft Preparation: **C.T.Y.** Writing – Review and Editing: **T.Y.** and **C.P.F.** Visualisation: **C.T.Y.** and **T.Y.** Supervision: **T.Y.** Project Administration: **T.Y.** Funding Acquisition: **T.Y.**

Declaration of interest

The authors declare no conflicts of interest.

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