Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation

R. A. Law,^{*1} F. J. Young,^{*} D. C. Patterson,^{*} D. J. Kilpatrick,[†] A. R. G. Wylie,[†] and C. S. Mayne^{*}

^{*}Agri-Food and Biosciences Institute, Agriculture Branch, Hillsborough, County Down, Northern Ireland BT26 6DR

[†]Agri-Food and Biosciences Institute, Newforge Lane, Belfast, Northern Ireland BT9 5PX

ABSTRACT

Ninety autumn-calving Holstein dairy cows [45 primiparous and 45 multiparous (mean parity, 3.1)] were allocated to 1 of 3 dietary crude protein (CP) concentrations: 173, 144, or 114 g of CP/kg of DM, from calving until d 150 of lactation. On d 151, half of the animals in each treatment were allocated an alternative dietary protein concentration. Half of the animals receiving 114 g of CP/kg of DM went onto 144 g of CP/kg of DM; half of the animals receiving 144 g of CP/kg of DM went onto 173 g of CP/kg of DM; and half of the animals receiving 173 g of CP/kg of DM went onto 144 g of CP/kg of DM, with the remaining animals staying on their original treatment. This resulted in 6 treatments in the mid to late lactation period: 114/114, 144/144, 173/173, 114/144, 144/173, and 173/144 g of CP/kg of DM. An increase in dietary CP concentration significantly increased milk, fat, and protein yield in early lactation (d 1 to 150). Dry matter intake was also increased with increased dietary protein concentration; however, this was not significant between 144 and 173 g of CP/kg of DM. Increased dietary CP significantly increased plasma urea, albumin, and total protein concentrations but had no significant effect on NEFA, leptin, or IGF-1 concentrations. Decreasing the dietary CP concentration in mid-late lactation (d 151 to 305) from 173 to 144 g/kg of DM had no significant effect on milk yield, dry matter intake, or milk fat and protein yield, compared with animals that remained on 173 g of CP/kg of DM throughout lactation. Increasing dietary CP concentration from 144 to 173 g/kg of DM significantly increased dry matter intake compared with animals that remained on the 144 g of CP/kg of DM throughout lactation. There were no significant dietary treatment effects on live weight or body condition score change throughout the experiment. Results of this study indicate that high protein diets (up to 173 g of CP/kg of DM) improved feed intake and animal performance

in early lactation (up to d 150), but thereafter, protein concentration can be reduced to 144 g of CP/kg of DM with no detrimental effects on animal performance.

Key words: dietary protein, milk production, dry matter intake, energy balance

INTRODUCTION

The ever increasing requirement to maximize the economic efficiency of animal production has driven forward the intensification of farming systems. The exponential increase in the genetic potential for milk production of the dairy cow has resulted in an increase in dietary CP concentration of diets to ensure a sufficient supply of MP to achieve maximal milk production. At present, for high-yielding dairy cows a CP level of approximately 180 g/kg of DM is commonly used in commercial diets (Kung, 2000). However, previous reports have suggested that increasing dietary protein concentration above 167 g/kg of DM has no benefit in terms of yield of milk or milk components (Broderick, 2003). High dietary CP levels are positively associated with the degradation of protein in the rumen (increased ammonia concentrations) and have been shown to decrease the efficiency of nitrogen utilization for milk production (Broderick, 2003; Hristov et al., 2004). From an environmental perspective, 0.65 to 0.75 of nitrogen consumed is excreted via urine and feces (Chase, 1994; Yan et al., 2006). Yan et al. (2006) showed that nitrogen excretion in manure was highly correlated with dietary nitrogen intake, and hence a key mitigation strategy to reduce manure nitrogen output is to reduce dietary nitrogen concentrations. Furthermore, previous studies have shown that an over-supply of rumen degradable protein (relative to fermentable ME supply) will increase the diffusion of ammonia from the rumen to the portal blood supply and subsequently increase urea production (detoxification by-product) in the liver (Roseler, 1994). In addition, ureagenesis (conversion of absorbed ammonia into urea in the liver) is less efficient in cows with fatty liver (Strang et al., 1998), which will reduce the detoxification of ammonia in the blood. The toxicity of ammonia has been reported to inhibit vital

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¹Corresponding author: Ryan.Law@afbini.gov.uk

processes such as gluconogenesis and the functionality of the tricarboxylic acid cycle (Rodriguez et al., 1997). Therefore, the balance between rumen degradable protein and fermentable carbohydrate is important in achieving an optimal ammonia concentration in the rumen.

The objective of this study was to investigate the effects of a range of dietary protein concentrations (120, 150, and 180 g/kg of DM) on animal production, energy metabolism, blood parameters, and nitrogen efficiency during early lactation (1 to 150 d), and the subsequent effects of altering dietary protein concentration at d 151 of lactation.

MATERIALS AND METHODS

Animals and Housing

Ninety Holstein-Friesian dairy cows [45 primiparous and 45 multiparous (mean parity 3.1)] were used in the experiment. Calving commenced on August 29 and ended on December 23. Following calving, animals were housed as a single unit in free stalls with concrete flooring. The cubicle to cow ratio was $\geq 1:1$ at all times, meeting the recommendations set by FAWC (1997). Cubicles had a bed measurement of 2.20 m long and 1.25 m wide, were fitted with rubber mats, and were bedded with sawdust thrice weekly. Concrete passage-way floors were scraped a minimum of 4 times daily using an automated system. Cows were milked twice daily at 0530 and 1630 h, with cows traveling about 35 m to the milking parlor. Lights were left on in the buildings at all times.

Experimental Design, Diets, and Feeding

The experiment involved allocating 90 freshly calved Holstein-Friesian dairy animals to 3 dietary treatments that differed in overall CP levels, as formulated, in the complete diet (DM basis); 180, 150, and 120 g/kg of DM. At d 151 of lactation, half of the animals in each treatment were allocated (balanced for parity, milk yield, calving date and live weight) an alternative dietary CP concentration, while the remaining animals were maintained on their original diets. Half of the animals receiving 120 g of CP/kg of DM went onto 150 g of CP/kg of DM; half of the animals receiving 150 g of CP/kg of DM went onto 180 g of CP/kg of DM; and half of the animals receiving 180 g of CP/kg of DM went onto 150 g of CP/kg of DM. This resulted in 6 treatments (1 to 151/151 to 305 DIM): 120/120, 150/150, 180/180, 120/150, 150/180, and 180/150. Primiparous animals were assigned in a balanced manner to treatments based on heifer rearing regime, calving date, and

live weight. Multiparous animals were assigned to treatments according to parity, previous lactation milk yield, calving date, and live weight. The diet was presented as a TMR, and animals were fed between 1000 and 1100 h daily using a diet feeder. Animals had free access to water at all times. The concentrate to forage ratio (DM basis) was 0.55:0.45 for all diets. The forage component of the diet consisted of 0.60 grass silage and 0.40 maize silage (DM basis). Samples of grass and maize silage were taken weekly and analyzed using near infrared reflectance spectroscopy (Park et al., 1998), and twice weekly for measurement of nitrogen and ammonia nitrogen using methods outlined by Steen (1989). Two concentrates were formulated to contain 229 and 117 g of CP/kg of DM to achieve target protein levels in the overall diets of 180 and 120 g of CP/kg of DM, respectively. Diets containing 150 g of CP/kg of DM were produced by complementing the forage component with equal amounts of concentrates containing 117 and 229 g of CP/kg of DM. The energy and protein concentrations of individual ingredients were based on published values (AFRC, 1993), which were used in the initial formulation of the concentrate proportion of the diet as presented in Table 1. Concentrate samples were taken weekly during the experiment and analyzed for DM, ash, ADF, NDF, and nitrogen as described by Cushnahan and Gordon (1995). The TMR diets were offered *ad libitum* using feed boxes that were placed on a computer-recorded load cell system, with controlled access to the boxes using an electronic identification system. This enabled DMI of individual animals to be recorded continuously via automatic feeding gates (Calan gate feeder), from which a daily intake was calculated and then averaged on a weekly basis.

Measurements

Milk yields were recorded daily at each milking throughout the experiment. Milk composition (fat, protein, lactose, and SCC) was determined on a weekly basis from one consecutive a.m. and p.m. milking. Separate analysis was completed for a.m. and p.m. samples, and milk composition was calculated on the basis of recorded a.m. and p.m. milk yields. Milk composition was determined using an infrared milk analyzer.

Live weight and BCS (scale: 0 to 5; Edmonson et al., 1989) were recorded on a weekly basis and throughout lactation. Animals were blood sampled once weekly, between 0930 and 1130 h, from the coccygeal vein using uncoated, heparin-coated, and fluoride oxalate-coated tubes (BD, Oxford, UK) from calving until d 100 of lactation, and then fortnightly thereafter. Plasma was recovered by centrifugation for analysis of glucose (fluoride oxalate tubes), total protein, albumin, globulin, BHBA,

Table 1. Ingredient formulation of concentrates (g/kg fresh) and energy and protein concentration (as formulated)

Item	Low protein (g/kg, DM)	High protein (g/kg, DM)
Barley (milled)	240	140
Wheat (milled)	240	140
Unmolassed sugar beat pulp	163	94
Citrus pulp	163	94
Maize gluten feed	30	100
Distillers grain (maize)	80	100
Soybean meal (Hi-Pro)	0	165
Rape meal	0	100
Megalac ¹	22	14
Trace minerals and vitamins	3	3
Salt	4	4
Dicalcium phosphate	12	0
Limestone (CaCO ₃)	15	15
Magnesium oxide	6	6
Molasses	24	24
ME (MJ/kg of DM)	13.1	13.1
CP (g/kg of DM)	116.6	228.6
Effective RDP (g/kg of DM)	78.5	134.8
Digestible undegradable protein (g/kg of DM)	28.9	73.5
Starch (g/kg of DM)	310.6	214.9

¹Volac Ltd., Orwell, Hertfordshire, UK.

NEFA, and urea (heparinized tubes), respectively, by clinical analyzer (Olympus UK Ltd., Middlesex, UK). Nonesterified fatty acid concentrations were determined using a standard kit (Wako Chemicals GmbH, Neuss, Germany). Uncoated tubes provided serum for leptin and IGF-1 RIA. Plasma and serum samples were stored at -20°C until analyzed. Radioimmunoassays were balanced for dietary treatments, and parity and control samples were included in each assay.

Serum leptin concentrations were determined in samples taken from all animals in each of wk 2, 4, 6, 8, 10, 12, 16, and 20. The primary anti-leptin antibody (OL-3) was raised in guinea pigs using recombinant ovine leptin kindly donated by A. Gertler (The Hebrew University of Jerusalem) and was included at a final dilution of 1:160,000 in each assay. Primary antiserum (100 µL) and plasma (100 µL) were incubated overnight (20 h) in a refrigerator after which was added 100 µL (12,000 to 15,000 cpm) of ¹²⁵I-iodinated ovine leptin, prepared using iodogen (Perbio Science, Northumberland, UK). Tubes were then held for a further 24 h in a refrigerator before addition of 100 µL of a cellulose-immobilized second antibody suspension (Sac-Cel anti-guinea pig IgG; IDS Ltd., Washington, Tyne on Wear, UK). Tubes were then left at room temperature for 20 min before addition of 1 mL of deionized water and centrifugation at 1,900 × *g* for 20 min. Following centrifugation, supernatant was removed, and radioactivity in each pellet was counted using a Cobra II gamma counter (Packard Canberra, Reading, UK).

Serum IGF-1 concentrations were determined in samples taken, in wk 2, 6, 10, 16 and 20, from 4 multiparous and 4 primiparous animals, randomly selected from each of the 3 dietary treatments. Insulin-like growth factor-1 binding proteins were removed by acid-ethanol cryo-precipitation (Wylie et al., 1997) before analysis for free IGF-1. The primary antibody (AFP 4892898) was rabbit anti-human somatomedin C (a gift from A. Parlow of the US National Hormone and Pituitary Program, Torrance, CA), used at a final dilution of 1:400,000. Human recombinant IGF-1 purchased from NIBSC (National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK) was used as the standard, and the label was ¹²⁵I-human recombinant IGF-1 (Amersham International, Buckinghamshire, UK) included at 12,000 to 15,000 cpm per tube. Bound IGF-1 label was recovered by addition of a second antibody (goat anti-rabbit IgG, Sigma Chemical Co, Poole, UK) followed by precipitation with polyethylene glycol succinate (6% wt/vol solution). After centrifugation and removal of supernatant, pellets were counted using a Cobra II gamma counter. Blood samples were not analyzed for leptin and IGF-1 from 151 to 305 DIM.

Calculation of Energy Balance

The average daily energy balance for each animal was calculated each week of lactation using the equations described by Thomas (2004) {energy balance = ME intake - ME requirement [-10 + (ME_{preg} + ME_{maintmilk} × Lwt^{0.75})] + [(0.0013 × Lwt)/K_m]; K_m, efficiency of energy use for maintenance [0.35 × (ME/GE) + 0.503]}. Daily milk yield, daily DMI, weekly milk composition, weekly live weight, and feed composition data were used in the calculations. The ME contents of grass and maize silage were obtained on a weekly basis using near infrared reflectance spectroscopy (Park et al., 1998), and ME contents of the concentrate were as formulated. Missing values were estimated from the week before and the week after missing data. Less than 2% of the data were missing.

Statistical Analysis

A repeated measures approach using the residual maximum likelihood procedure available in GenStat (Payne et al., 1993) was used to analyze the data set. The model fitted fixed effects for parity, dietary protein treatment, and stage of lactation (weeks from calving) for each parameter. The model included all 2-level interactions between these variables. Additional orthogonal contrasts were calculated for linear and quadratic effects of treatments from 1 to 150 DIM. There was

Table 2. Composition of TMR as fed, indicating DM, CP, and ME contents

Item	Target CP concentration (g/kg of DM)								
	180			150			120		
	Concentrate	Grass silage	Maize silage	Concentrate	Grass silage	Maize silage	Concentrate	Grass silage	Maize silage
Proportion in diet	0.55	0.27	0.18	0.55	0.27	0.18	0.55	0.27	0.18
DM content (g/kg)	870	343	266	870	343	266	870	343	266
CP (g/kg of DM)	217	147	78.6	163.5	147	78.6	110	147	78.6
ME (MJ/kg of DM)	13.1	11.9	10.8	13.1	11.9	10.8	13.1	11.9	10.8
Total ME (MJ/kg of DM)		12.4			12.4			12.4	
Total CP (g/kg of DM)		173			144			114	
ERDP ¹ (g/kg of DM)		112.4			94.5			75.5	
DUP ¹ (g/kg of DM)		62.6			48.2			34.7	

¹ERDP = effective RDP; DUP = digestible undegradable protein.

no significant effect of dietary treatment on condition score change or live weight over the first 3 wk of lactation. Therefore, the deviation from the mean of these 2 variables, in multi- and primiparous animals during this period, was used as a covariate. Due to the design of this experiment, data were analyzed in 3 parts: 1 to 150 DIM, 151 to 305 DIM, and 1 to 305 DIM. An additional covariate was included in the analysis of data from 151 to 305 DIM: the difference between the mean of the variable in question from 135 to 150 DIM for each animal, and the overall mean of the same variable from animals within the same treatment from 135 to 150 DIM.

RESULTS

Table 2 illustrates the composition (as fed) of diets targeted to contain 120, 150 and 180 g of CP/kg of DM. Actual values were 114, 144, and 173 g of CP/kg of DM respectively. All diets were isoenergetic. The effective rumen degradable protein [eRDP, estimated using Feed into Milk (Thomas, 2004)] to digestible undegradable protein (DUP) ratios for diets containing 114, 144, and 173 g of CP/kg of DM were 2.17, 1.96, and 1.79, respectively [Feedbyte (Feed into Milk; Thomas, 2004)].

Production Responses to Dietary Protein in Early and Mid Lactation (1 to 150 DIM)

Dietary protein concentration effects on DMI, ME intake, milk yield, milk constituents (fat, protein, and lactose), constituent yields (fat and protein), and milk energy output are presented in Table 3. Increased dietary protein concentration significantly ($P < 0.001$) increased milk yield, milk protein concentration, total milk fat yield, total milk protein yield, and milk energy

output. Increasing dietary protein concentration from 114 to 144/173 g/kg of DM significantly increased DMI ($P < 0.001$) and decreased milk fat concentration ($P < 0.05$). There was no significant ($P > 0.05$) difference in DMI or milk fat concentration between animals receiving 144 and 173 g of CP/kg of DM. However, orthogonal contrasts for linear and quadratic effects suggest that the relationship between dietary protein concentration and DMI, and milk fat concentrations is linear ($P < 0.001$ and $P < 0.05$, respectively).

During the first 150 d of lactation, an increase in dietary protein content significantly ($P < 0.001$) increased the average daily ME requirement (Table 4). Increasing dietary protein concentration from 114 to 144/173 g/kg of DM significantly increased ME intake and live weight. There was no significant difference in ME intake or live weight of animals between 144 and 173 g of CP/kg of DM. Orthogonal contrasts for linear and quadratic effects suggest that the relationship between dietary protein concentration and live weight is curvilinear [significant ($P < 0.01$) linear and quadratic effects]. Increasing dietary protein concentration from 114 to 173 g/kg of DM significantly ($P < 0.001$) decreased daily energy balance. However, there was no significant difference in daily energy balance between animals on 144 g of CP/kg of DM and those on 114 and 173 g of CP/kg of DM. Increasing dietary protein concentration from 114/144 to 173 g/kg of DM significantly decreased cumulative energy balance. There was no significant effect of dietary protein concentration on the change in live weight or BCS over the first 150 DIM. There was a significant ($P < 0.05$) effect of dietary protein concentration on the interval to energy nadir (cumulative energy balance at lowest point). Dietary protein concentrations of 114, 144, and 173 g/kg of DM produced intervals to energy nadir of 7.8, 10.8, and 14.8 wk (SED = 2.61), respectively.

Table 3. Effects of dietary protein concentration on DMI, milk yield, milk constituents (fat, protein, and lactose), and constituent yields (fat and protein) of animals in early and mid lactation (1 to 150 d)

Item	Dietary CP concentration (g/kg of DM)			SED ¹	P-value	Contrast	
	114	144	173			Linear	Quadratic
DMI (kg/d)	16.5 ^b	18.0 ^a	18.6 ^a	0.35	***	***	NS
Milk yield (kg/d)	25.4 ^c	31.8 ^b	35.4 ^a	1.14	***	***	NS
Milk constituents (g/kg)							
Fat	42.0 ^a	38.3 ^b	38.1 ^b	1.40	*	*	NS
Protein	31.4	32.3	32.4	0.61	NS	NS	NS
Lactose	48.2	48.1	47.8	0.46	NS	NS	NS
Milk constituent yield (kg/d)							
Fat	1.07 ^c	1.21 ^b	1.35 ^a	0.058	***	***	NS
Protein	0.80 ^c	1.03 ^b	1.15 ^a	0.031	***	***	*
Fat + protein	2.15 ^c	2.41 ^b	2.70 ^a	0.115	***	***	NS
Milk energy output (MJ/d)	80.4 ^c	96.2 ^b	107.2 ^a	3.52	***	***	NS

^{a-c}Values within a row with no common superscript differ ($P < 0.05$).

¹SED = standard error of the difference.

NS, $P > 0.05$; * $P < 0.05$; *** $P < 0.001$.

An increase in the dietary protein concentration significantly ($P < 0.001$) reduced the efficiency of nitrogen use for milk production (Table 5). From 1 to 150 DIM animals receiving 114 g of CP/kg of DM had a nitrogen efficiency of 0.423 in comparison with 0.350 in animals receiving 173 g of CP/kg of DM.

Blood Responses to Dietary Protein in Early and Mid Lactation (1 to 150 DIM)

The effects of dietary protein concentration on blood parameters are presented in Table 6. An increase in dietary protein concentration significantly increased plasma urea ($P < 0.001$), total protein ($P < 0.001$) and albumin ($P < 0.001$) concentrations, and decreased plasma BHBA ($P < 0.001$) concentrations. An orthogonal contrast for linear and quadratic effects identified a curvilinear relationship between urea and dietary CP concentration [significant linear ($P < 0.001$) and qua-

dratic trends ($P < 0.01$)]. There was no significant ($P > 0.05$) effect of dietary protein concentration on serum IGF-1, leptin, plasma glucose, globulin, or NEFA concentrations.

Significant effects of parity were also realized for blood parameters. Primiparous animals had significantly less albumin (32.3 vs. 33.2; $P < 0.05$), total protein (76.4 vs. 78.7; $P < 0.01$), and urea concentrations (2.70 vs. 2.95; $P < 0.01$) than multiparous animals.

Dietary Protein Adjustment at d 151 of Lactation

The effects of adjusting dietary protein concentration at d 151 of lactation on production parameters are presented in Tables 7 and 8. From d 151 to 305 of lactation, increasing dietary protein concentration from 114 to 144 g/kg of DM significantly increased DMI ($P < 0.001$), ME intake ($P < 0.001$), milk yield ($P < 0.001$), milk fat yield ($P < 0.001$), milk protein yield ($P <$

Table 4. Effects of dietary protein concentration on live weight, BCS, and body energy status of animals in early and mid lactation (1 to 150 d)

Item	Dietary CP concentration (g/kg of DM)			SED ¹	P-value	Contrast	
	114	144	173			Linear	Quadratic
Live weight (kg)	531.5 ^b	548.3 ^a	544.0 ^a	4.18	***	**	**
Live weight gain (kg/d)	0.16	0.27	0.24	0.193	NS	NS	NS
BCS	2.38	2.41	2.37	0.030	NS	NS	NS
BCS change (units/wk)	-0.004	0.006	0.001	0.007	NS	NS	NS
ME requirement (MJ/d)	191.3 ^c	222.3 ^b	242.3 ^a	6.34	***	***	NS
ME intake (MJ/d)	204.2 ^b	222.8 ^a	231.0 ^a	4.54	***	***	NS
Daily energy status (MJ/d)	12.78 ^a	0.539 ^{ab}	-11.05 ^b	6.20	**	***	NS
Cumulative energy status (MJ)	414 ^a	-537 ^a	-1,801 ^b	520	***	***	NS

^{a-c}Values within a row with no common superscript differ ($P < 0.01$).

¹SED = standard error of the difference.

NS, $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 5. Effect of altering dietary protein concentration at d 151 of lactation on the efficiency of nitrogen use for milk production from d 1 to 150 and 151 to 305

Item	Dietary CP concentration (g/kg of DM)			SED ¹	P-value	Contrast		
	114	144	173			Linear	Quadratic	
Day 1 to 150								
Dietary nitrogen intake (kg)	0.300 ^c	0.414 ^b	0.515 ^a	0.0059	***	***	NS	
Milk nitrogen output (kg)	0.126 ^c	0.160 ^b	0.179 ^a	0.0020	***	***	***	
Efficiency of nitrogen use for milk production	0.423 ^a	0.391 ^b	0.350 ^c	0.0048	***	***	NS	
	Dietary CP concentration ²							
	114/114	144/144	173/173	114/144	144/173	173/144	SED	P-value
Day 151 to 305								
Dietary nitrogen intake (kg)	0.307 ^e	0.411 ^d	0.535 ^b	0.414 ^d	0.545 ^a	0.433 ^c	0.0043	***
Milk nitrogen output (kg)	0.120 ^d	0.151 ^b	0.161 ^a	0.135 ^c	0.163 ^a	0.155 ^b	0.0025	***
Efficiency of nitrogen use for milk production	0.390 ^a	0.368 ^b	0.300 ^d	0.326 ^c	0.299 ^d	0.360 ^b	0.0049	***

^{a-e}Values within a row with no common superscript differ ($P < 0.001$).

¹SED = standard error of the difference.

²The first value is the dietary CP concentration for d 1 to 150; the second value is the CP concentration for d 151 to 305.

NS, $P > 0.05$; *** $P < 0.001$.

0.001), and fat plus protein yield ($P < 0.001$). Increasing dietary protein concentration from 144 to 173 g/kg of DM significantly increased DMI ($P < 0.001$), ME intake ($P < 0.001$), milk fat yield ($P < 0.001$), milk protein yield ($P < 0.001$), and fat plus protein yield ($P < 0.001$). There was no significant effect ($P > 0.05$) of increasing dietary protein concentration from 144 to 173 g/kg of DM on milk yield during the second half of lactation. Decreasing dietary protein concentration from 173 to 144 g/kg of DM had no significant effect ($P > 0.05$) on DMI, ME intake, milk yield, milk fat yield, milk protein yield, or fat plus protein yield. There was no significant effect ($P > 0.05$) of dietary protein change on the concentration of milk fat, protein, or lactose.

Data presented in Table 8 illustrates that increasing dietary protein concentration from 114 to 144 g/kg of DM at d 151 of lactation significantly increased ME requirement ($P < 0.001$) and significantly decreased

cumulative energy balance ($P < 0.001$), compared with animals that remain on 114 g of CP/kg of DM throughout lactation. There was no significant effect ($P > 0.05$) of increasing dietary protein concentration from 114 to 144 g/kg of DM on live weight or daily energy balance. An increase in the dietary protein concentration from 144 to 173 g/kg of DM significantly increased live weight ($P < 0.01$), ME requirement ($P < 0.001$), and daily energy balance ($P < 0.01$). There was no significant effect ($P > 0.05$) of altering the protein concentration of the diet from 144 to 173 g/kg of DM on cumulative energy balance. Additionally, there was no significant effect ($P > 0.05$) of decreasing dietary protein concentration from 173 to 144 g/kg of DM on live weight, ME requirement, daily energy balance, or cumulative energy balance. Dietary protein concentration had no significant effect on live weight change, BCS, or BCS change.

Table 6. Effects of dietary protein concentration on blood constituents of animals in early and mid lactation (1 to 150 d)

Blood parameter	Dietary CP concentration (g/kg of DM)			SED ¹	P-value	Contrast	
	114	144	173			Linear	Quadratic
Urea (mmol/L)	1.56 ^c	2.59 ^b	4.32 ^a	0.130	***	***	**
Total protein (g/L)	75.5 ^c	77.0 ^b	80.2 ^a	1.01	***	***	NS
Albumin (g/L)	31.2 ^b	33.3 ^a	33.7 ^a	0.53	***	***	NS
Globulin (g/L)	44.3	43.7	46.5	1.31	NS	NS	NS
NEFA (mEq/L)	0.386	0.368	0.402	0.029	NS	NS	NS
BHBA (mmol/L)	0.558 ^a	0.499 ^{ab}	0.476 ^b	0.031	*	**	NS
Glucose (mmol/L)	3.33	3.32	3.34	0.044	NS	NS	NS
Leptin ² (ng/mL)	1.97	2.04	2.00	0.352	NS	NS	NS
IGF-1 ² (ng/mL)	103	103	99	6.5	NS	NS	NS

^{a-c}Values within a row with no common superscript differ ($P < 0.05$).

¹SED = standard error of the difference.

²IGF-1 samples ($n = 24$; 5 time points), leptin samples ($n = 72$; 8 time points), and all other parameters ($n = 90$; 16 time points).

NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 7. Effect of altering dietary protein concentration at d 151 of lactation on DMI, milk yield, milk constituents (fat, protein, and lactose) milk constituent yields (fat and protein), and milk energy output of animals from d 151 to 305

Item	Dietary CP concentration ¹ (g/kg of DM)						SED ²	P-value
	114/114	144/144	173/173	114/144	144/173	173/144		
DMI (kg/d)	16.8 ^d	17.8 ^c	19.3 ^{ab}	18.0 ^{bc}	19.7 ^a	18.7 ^b	0.45	***
Milk yield (kg/d)	23.0 ^c	28.8 ^a	29.8 ^a	26.3 ^b	30.7 ^a	29.8 ^a	1.07	***
Milk constituents (g/kg)								
Fat	42.3 ^a	38.3 ^{bc}	38.1 ^{bc}	42.7 ^a	39.8 ^b	37.4 ^c	0.99	***
Protein	33.2	33.5	33.7	32.6	33.7	33.5	0.60	NS
Lactose	48.4 ^a	48.0 ^{ab}	47.1 ^b	48.9 ^a	48.6 ^a	48.0 ^{ab}	0.53	*
Milk constituent yield (kg/d)								
Fat	0.97 ^c	1.10 ^b	1.13 ^{ab}	1.11 ^b	1.21 ^a	1.13 ^{ab}	0.046	***
Protein	0.76 ^d	0.94 ^b	1.03 ^a	0.86 ^c	1.04 ^a	0.99 ^{ab}	0.036	***
Fat + protein	1.93 ^c	2.20 ^b	2.25 ^b	2.21 ^b	2.43 ^a	2.27 ^{ab}	0.092	***
Milk energy output (MJ/d)	72.2 ^c	87.8 ^{ab}	89.5 ^a	81.7 ^b	94.8 ^a	89.1 ^a	3.57	***

^{a-d}Values within a row with no common superscript differ ($P < 0.05$).

¹The first value is the dietary CP concentration for d 1 to 150; the second value is the CP concentration for d 151 to 305.

²SED = standard error of the difference.

NS, $P > 0.05$; * $P < 0.05$; *** $P < 0.001$.

Data presented in Table 5 indicate that the efficiency of nitrogen use for milk production was significantly ($P < 0.001$) greater in animals that were changed from 173 to 144 g of CP/kg of DM at d 151 of lactation in comparison with those that remained on 173 g of CP/kg of DM throughout lactation.

The effects of changing dietary protein concentration at d 151 of lactation on blood parameters are presented in Table 9. Increasing the dietary CP concentration from 114 to 144 g/kg of DM at d 151 of lactation significantly increased the concentrations of urea ($P < 0.001$) and total protein ($P < 0.01$) from 151 to 305 DIM. Increasing the dietary concentration from 144 to 173 g/kg of DM at d 151 of lactation significantly ($P < 0.001$) increased the concentration of urea. Decreasing the dietary protein concentration from 173 to 144 g/kg of DM at d 151 of lactation significantly ($P < 0.001$) decreased the concentration of urea.

Production Responses from 1 to 305 DIM

When evaluated over a full 305 d lactation, there were no significant ($P > 0.05$) differences in DMI, milk yield, milk fat concentration, milk fat yield, milk protein yield, milk fat plus protein yield, milk energy output, daily energy balance, or cumulative energy balance in animals receiving 173 g of CP/kg of DM throughout lactation and those receiving 173 g of CP/kg of DM from 1 to 150 DIM and 144 g of CP/kg of DM from 151 to 305 DIM (Table 10). Animals receiving 144 g of CP/kg of DM throughout lactation had significantly less ($P < 0.001$) DMI than animals receiving 144 g of CP/kg of DM from 1 to 150 DIM and 173 g of CP/kg of DM from 151 to 305 DIM (Table 10). However, there was no significant difference in milk yield, milk fat concentration, milk fat yield, milk protein yield, milk fat plus protein yield, milk energy output, daily energy balance,

Table 8. Effect of altering dietary protein concentration at d 151 of lactation on live weight, BCS, and body energy status of animals from d 151 to 305

Item	Dietary CP concentration ¹ (g/kg of DM)						SED ²	P-value
	114/114	144/144	173/173	114/144	144/173	173/144		
Live weight (kg)	573 ^b	579 ^b	569 ^b	578 ^b	593 ^a	570 ^b	6.41	**
Live weight gain (kg/d)	0.47	0.48	0.30	0.49	0.46	0.43	0.078	NS
BCS	2.66	2.60	2.53	2.57	2.65	2.53	0.078	NS
BCS change (units/wk)	0.012	0.017	0.006	0.011	0.014	0.008	0.007	NS
ME requirement (MJ/d)	179 ^c	205 ^{ab}	203 ^b	197 ^b	217 ^a	204 ^b	6.21	***
ME intake (MJ/d)	209 ^d	218 ^{bc}	232 ^b	221 ^c	244 ^a	226 ^{bc}	5.79	***
Daily energy status (MJ/d)	31.2 ^a	13.2 ^b	29.3 ^a	20.1 ^{ab}	28.2 ^a	21.5 ^{ab}	5.76	**
Cumulative energy status (MJ)	4,218 ^a	987 ^{cd}	716 ^d	3,202 ^b	1,790 ^c	27 ^d	482	***

^{a-d}Values within a row with no common superscript differ ($P < 0.01$).

¹The first value is the dietary CP concentration for d 1 to 150; the second value is the CP concentration for d 151 to 305.

²SED = standard error of the difference.

NS, $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 9. Effect of altering dietary protein concentration at d 151 of lactation on blood constituents of animals from d 151 to 305

Item	Dietary CP concentration ¹ (g/kg of DM)						SED ²	P-value
	114/114	144/144	173/173	114/144	144/173	173/144		
Urea (mmol/L)	2.06 ^d	3.29 ^c	5.43 ^a	3.43 ^c	5.13 ^a	4.16 ^b	0.303	***
Total protein (g/L)	77.2 ^c	80.2 ^b	82.6 ^a	80.4 ^b	79.0 ^{bc}	81.1 ^{ab}	1.45	**
Albumin (g/L)	33.3	34.2	34.2	34.4	34.9	34.5	0.60	NS
Globulin (g/L)	43.9	45.8	48.3	46.0	44.3	46.6	1.68	NS
NEFA (mEq/L)	0.27	0.29	0.30	0.32	0.24	0.39	0.045	NS
BHBA (mmol/L)	0.55	0.51	0.47	0.53	0.53	0.51	0.50	NS
Glucose (mmol/L)	3.31	3.20	3.32	3.30	3.26	3.23	0.078	NS

^{a-d}Values within a row with no common superscript differ ($P < 0.01$).

¹The first value is the dietary CP concentration for d 1 to 150; the second value is the CP concentration for d 151 to 305.

²SED = standard error of the difference.

NS, $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$.

or cumulative energy balance between these 2 groups. Animals receiving 114 g of CP/kg of DM throughout lactation had a significantly ($P < 0.001$) greater daily energy balance and lesser milk yields, milk fat yields, milk protein yields, milk fat plus protein yield, and milk energy outputs than animals receiving 114 g of CP/kg of DM from 1 to 150 DIM and 144 g of CP/kg of DM from 151 to 305 DIM (Table 10). There was no significant difference in DMI, milk fat concentration, or cumulative energy balance between these 2 groups.

DISCUSSION

Dietary Protein Effects on Production Parameters

Increasing dietary CP concentrations (1 to 150 DIM) from 114 to 144 and from 144 to 173 g/kg of DM realized daily milk yield responses of 6.4 and 3.6

kg/d, respectively. Despite these differences suggesting a curvilinear relationship, results from an orthogonal contrast for linear and quadratic effects state that only a linear relationship exists (Table 3). These results are in contrast to previous authors who have demonstrated a quadratic relationship between milk output and dietary protein intake. Olmos Colmenero and Broderick (2006) reported an increase in milk yield, with increasing dietary CP concentration up to 165 g/kg of DM, after which point a decline in milk yield was noted. They also noted a similar pattern of increase in milk protein and fat yields. Furthermore, Cunningham et al. (1996) and Leonardi and Armentano (2003) observed no improvement in milk yield when dietary CP increased from 165 to 185 g/kg of DM and from 161 to 189 g/kg of DM, respectively. In the present study, Figure 1 demonstrates a tendency toward a greater milk yield response when increasing dietary protein concentration

Table 10. Effect of altering dietary protein concentration at d 151 of lactation on DMI, milk yield, milk constituents (fat, protein, and lactose), milk constituent yields (fat and protein), milk energy output, and daily and cumulative energy balance of animals from d 1 to 305

Item	Dietary CP concentration ¹ (g/kg of DM)						SED ²	P-value
	114/114	144/144	173/173	114/144	144/173	173/144		
DMI (kg/d)	16.8 ^d	17.8 ^{bc}	18.6 ^{ab}	17.5 ^{cd}	18.9 ^a	18.9 ^a	0.45	***
Milk yield (kg/d)	23.0 ^d	29.8 ^{bc}	33.0 ^a	26.9 ^c	30.0 ^b	30.7 ^{ab}	1.49	***
Milk constituents (g/kg)								
Fat	42.5 ^a	39.3 ^{ab}	36.6 ^b	42.0 ^a	38.6 ^b	40.4 ^{ab}	1.93	*
Protein	32.6	33.3	32.9	32.2	32.9	33.7	0.75	NS
Lactose	48.0	47.9	47.2	48.9	48.4	48.2	0.61	NS
Milk constituent yield (kg/d)								
Fat	0.97 ^b	1.17 ^a	1.20 ^a	1.13 ^a	1.12 ^a	1.23 ^a	0.067	**
Protein	0.74 ^d	0.99 ^b	1.08 ^a	0.86 ^c	0.98 ^b	1.03 ^{ab}	0.044	***
Fat + protein	1.94 ^b	2.33 ^a	2.40 ^a	2.27 ^a	2.24 ^a	2.46 ^a	0.135	**
Milk energy output (MJ/d)	73.8 ^c	93.7 ^{ab}	98.8 ^a	86.0 ^b	91.7 ^{ab}	97.8 ^a	4.37	***
Daily energy balance (MJ/d)	27.4 ^a	4.40 ^b	4.65 ^b	12.1 ^b	18.0 ^{ab}	11.3 ^b	7.21	*
Cumulative energy balance (MJ)	2,870 ^a	-465 ^{bc}	-1,531 ^c	1,067 ^{ab}	1,788 ^{ab}	200 ^{bc}	1,145	**

^{a-d}Values within a row with no common superscript differ ($P < 0.05$).

¹The first value is the dietary CP concentration for d 1 to 150; the second value is the CP concentration for d 151 to 305.

²SED = standard error of the difference.

NS, $P > 0.05$; * $P < 0.05$; *** $P < 0.001$.

Figure 1. Effect of dietary protein concentration on milk production (legend: dietary protein concentration, 1 to 150 DIM/151 to 305 DIM).

from 114 to 144 g/kg of DM than from 144 to 173 g/kg of DM, especially in the early to mid lactation period. Figure 1 also indicates that post d 151 of lactation, there is a major decline in the efficiency of nitrogen utilization for milk production in animals allocated 173 g of CP/kg of DM throughout lactation (1 to 305 DIM), compared with those allocated a reduced dietary CP concentration (173 to 144 g/kg of DM) at d 151 of lactation. Animals receiving 173 g of CP/kg of DM before d 151 of lactation showed no detrimental effects on production when allocated 144 g of CP/kg of DM at d 151 of lactation. These conclusions are verified in Table 5, which illustrates that the nitrogen efficiency significantly increases when animals are reduced from 173 to 144 g of CP/kg of DM at d 151 of lactation.

Animals receiving 114 g of CP/kg of DM had significantly higher milk fat concentrations in comparison to animals on 144/173 g of CP/kg of DM during the first 150 d of lactation. However, orthogonal contrast for linear and quadratic effects identified a linear relationship between these 2 variables. These results are in contrast to those reported by Leonardi and Armentano (2003; 161 to 189 g of CP/kg of DM) and M'Hamed et al. (2001; 141 to 170 g of CP/kg of DM) who found that milk fat concentration increased in response to dietary CP. Additionally, Lundquist et al. (1986) found no effect of altering dietary protein concentration on milk fat content. The latter authors had similar protein levels to those used in the current experiment (125, 155, and 180 g of CP/kg of DM). Total milk fat and protein

yields increased with increasing dietary protein concentrations, which is in partial agreement with Broderick (2003). Broderick (2003) stated that yields of fat and protein increased with increases in the concentration of dietary CP from 151 to 167 g/kg of DM but showed no further increase when dietary CP concentrations were increased to 184 g/kg of DM. In the current experiment, a linear response was realized between milk fat yield and the concentration of dietary CP. When considering total milk protein yield, Leonardi and Armentano (2003) found that it was unaffected by an increase in dietary CP content (161 to 189 g/kg of DM), with milk protein concentration decreasing. However, in the present study each increment in dietary CP produced a significant increase in milk protein yield; this relationship was curvilinear.

Broderick (2003) reported a linear increase in DMI with increases in dietary CP concentration from 152 to 167 and 183 g/kg of DM. A linear relationship was also identified in the current study; however, there was no significant difference in DMI (1 to 150 DIM) between animals receiving 144 and those receiving 173 g of CP/kg of DM. If fermentable energy supply is not a limiting factor, then increasing the amount of eRDP should realize an increase in microbial protein synthesis and generate increased dietary intakes because of increased microbial activity in the rumen. In the present study, the eRDP concentration of diets containing 114, 144, and 173 g of CP/kg of DM were predicted to be 75.5, 94.5, and 112.5 g/kg of DM, respectively. However, di-

Figure 2. Effect of changing dietary protein concentration on DMI (legend: dietary protein concentration, 1 to 150 DIM/151 to 305 DIM).

etary intake responses were not in proportion to eRDP increments, which would suggest that either the supply of fermentable energy was limiting, or that bacterial growth was approaching a maximum. In agreement with this, Cunningham et al. (1996) found no significant difference in DMI when animals were offered 165 and 185 g of CP/kg of DM. M'Hamed et al. (2001) concluded that increasing the protein content of the diet enhances DMI, milk yield, and BW gain, but that the responses vary greatly according to the type and level of protein supplementation.

Increasing dietary protein concentration from 114 to 173 g/kg of DM significantly reduced the daily and cumulative energy balance. Increasing dietary protein concentration from 114 to 173 g/kg of DM produced a 13.1% increase in ME intake but a 26.7% increase in ME requirement, which accounts for the decrease in daily energy balance. Additionally, an increase in dietary protein concentration from 114 to 173 g/kg of DM significantly increased the interval to energy nadir. Animals that display a more negative energy balance will take longer to reach an energetic equilibrium (zero energy balance). Results presented in Table 4 indicate that the observed variation in cumulative energy balance between animals receiving 114 and 173 g of CP/kg of DM is not reflected in BCS change or live weight. A difference in cumulative energy balance of 2,215 MJ (414 to -1,801) would equate to a difference in BCS of 1.25 units (1,770 MJ/unit BCS loss; AFRC, 1993), and a difference in live weight of 95 kg [23.4 MJ/kg of body live weight loss (AFRC, 1990)]. The actual dif-

ference in BCS and live weight was 0.01 units and 12.5 kg (greater live weight in animals with more negative energy balance), respectively. These discrepancies may be partially a result of some basic assumptions used in the energy calculations. These assumptions include

1. The maintenance requirement of all animals is a constant value that is relative to metabolic BW, irrespective of the level of production.
2. The net efficiency of energy utilization for lactation (k_l), despite being partially scaled to level of intake (Thomas, 2004), does not go below 0.59.

Dietary Protein Effects on Blood Parameters (1 to 150 DIM)

Plasma urea and total protein concentrations were significantly elevated by an increase in dietary protein content. Increased plasma urea concentrations indicate increased ammonia detoxification in the liver, whereas an increase in total blood protein concentrations indicate intestinal absorption of protein, which will be evident at greater dietary protein contents (where fermentable carbohydrate is not limiting). Increased ammonia concentrations in the blood may be caused by an oversupply of eRDP in the rumen (Kenny et al., 2001). The curvilinear relationship identified between urea and dietary CP concentration would support this suggestion. There was no significant effect of dietary protein content on plasma NEFA, glucose, or globulin concentrations, which is in agreement with results re-

Figure 3. Effect of changing dietary protein concentration on cumulative energy balance (legend: dietary protein concentration, 1 to 150 DIM/151 to 305 DIM).

ported by M'Hamed et al. (2001). The latter authors showed no significant effects of increasing dietary protein concentration from 141 to 165 g of CP/kg of DM on concentrations of these blood parameters. Additionally, there were no significant effects of altering the dietary protein content on serum leptin or IGF-1 concentrations. Adipose tissue is the biggest contributor to plasma leptin in ruminants (Chilliard et al., 2001) and is positively correlated with body condition (Kokkonen et al., 2005). As there were no significant effects of dietary CP concentration on BCS, or BCS change in the current study (Tables 4 and 8), no effects on leptin would be expected. Similarly, Armstrong et al. (2001) found no effect of dietary protein on plasma IGF-1 concentrations.

Dietary Protein Effects on Production Parameters (151 to 305 DIM)

Data presented in Figure 1 indicate that reducing the dietary protein concentration at d 151 of lactation from 173 to 144 g/kg of DM had no detrimental effect on milk yield compared with animals that remained on 173 g of CP/kg of DM (Table 7). This would imply that reducing the dietary protein concentration of the diet post 150 DIM is a key mitigation strategy to improve the efficiency of nitrogen use for milk production. Animals receiving 114 g of CP/kg of DM from d 1 to 150 of lactation displayed significant increases in milk yield when allocated 144 g of CP/kg of DM during the

second part of lactation (151 to 305 DIM). There was also a trend (though not significant, $P > 0.05$) for an increase in milk yield of 1.9 kg per d (d 151 to 305) in animals changed from 144 to 173 g of CP/kg of DM at d 151, compared with those that remained on 144 g of CP/kg of DM.

Increasing protein concentrations from 114 to 144 and 144 to 173 g of CP/kg of DM at d 151 of lactation produced significant (Table 7; Figure 2) increases in DMI of 7.1 and 10.6%, respectively (Figure 2). Reducing protein concentration from 173 to 144 g of CP/kg of DM tended ($P > 0.05$) to decrease DMI (Table 7). Therefore, increasing dietary protein concentration from 114 to 144 g/kg of DM produced a 7.1% increase in ME intake but a 14.3% increase in milk yield, which resulted in a decrease in cumulative energy balance (Figure 3). Increasing dietary protein concentration from 144 to 173 g/kg of DM produced a 10.6% increase in ME intake but a 6.5% increase in milk yield resulting in a significantly greater energy balance.

CONCLUSIONS

The present study assessed the effects of altering dietary protein concentration on production parameters of lactating dairy cows. The results indicate that in early lactation (d 1 to 150) an increase in dietary protein content up to 173 g/kg of DM has beneficial effects on milk yield and DMI. However, in late lactation (151 to 305 DIM), more efficient use of dietary nitrogen can

be achieved by feeding diets with lesser dietary protein concentrations (between 144 and 173 g of CP/kg of DM) without realizing detrimental effects on production. Improving efficiency and economic sustainability of dairy cow production systems, as well as reducing the environmental impact of intensive dairy farming is critical, and these results indicate that reducing dietary protein concentration in mid/late lactation improves the efficiency of use of dietary nitrogen with no detrimental effects on animal performance.

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