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Production performance of Holstein cows at 4 stages of lactation fed 4 dietary crude protein concentrations

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ABSTRACT

Dairy cow responses to dietary crude protein (CP) may depend on stage of lactation. The primary objective of this study was to evaluate the effects of 4 concentrations of dietary CP on dry matter intake (DMI), production performance, net energy for lactation (NE_L) output in milk, feed efficiency (FE: milk NE_L/DMI), and nitrogen use efficiency (100 × milk protein-N/N intake) when fed to cows grouped as early, mid-early, mid-late, and late lactation. Our secondary objective was to determine the range of CP concentration at which production responses were not negatively affected across days in milk (DIM). Multiparous Holstein cows (n = 64) were stratified by DIM [initial average ± standard deviation: 86 ± 14.9 (early), 119 ± 10.0 (mid-early), 167 ± 22.2 (mid-late), and 239 ± 11.1 (late)] and then randomly assigned within DIM group to receive 1 of 4 total mixed rations containing 13.6, 15.2, 16.7, and 18.3% CP (dry matter basis) according to a 4 × 4 factorial arrangement of treatments. Cows were individually fed a covariate diet for 14 d, followed by 56 d of treatment diets. Milk yield and DMI were recorded daily and milk components were analyzed weekly for 2 consecutive days at 3 daily milkings. Data were analyzed using a categorical mixed-effect model to evaluate the effects of CP concentration and DIM using linear, quadratic, and cubic contrasts, and their interactions. Additionally, a mixed-effect cubic regression model was fit with DIM, dietary CP concentration, and their interaction as continuous independent variables. Dietary CP concentration deemed optimal across DIM was determined as the range of CP for which the dependent responses did not differ from the predicted maximum. With advancing stage of lactation, DMI, milk NE_L output, and FE decreased linearly (from 30.4 to 28.4 kg/d for DMI, from 33.2 to 23.3 Mcal/d for NE_L output, and from 1.09 to 0.82 Mcal milk NE_L/kg DMI for FE for early and late lactation cows, respectively). Responses to dietary CP concentration were linear, quadratic, and cubic with the greatest values observed when cows were fed the 16.7% CP diet across DIM (30.8 kg/d, 31.0 Mcal/d, and 1.01 Mcal/kg for DMI, milk NE_L output, and FE, respectively). There was an interaction between dietary CP concentration and stage of lactation for DMI, milk NE_L output, milk component yield, and FE, which was due to the decline in response to additional CP as lactation progressed. Compared with the 16.7% CP diet, feeding the 18.3% CP diet decreased milk NE_L 0.81 and 5.3 Mcal/d for early and late lactation cows, respectively, indicating that feeding a higher CP concentration in late lactation had a negative effect on cow performance. Nitrogen use efficiency declined linearly with increasing CP concentration and DIM. Regression analysis suggested that dietary CP ranging from 16.3 to 17.4% maintained production in early and mid-early lactation. However, dietary CP could be reduced to between 15.7 and 17.1% in late lactation. This research suggested that there are distinct ranges of dietary CP concentrations that maintain cow performance at each stage of lactation.

Key words: crude protein, stage of lactation, nitrogen use efficiency, performance

INTRODUCTION

Exceeding dairy cow dietary MP requirements results in reduced N use efficiency (**NUE**; milk true protein-N:N intake) because excess N is mainly lost in urine instead of contributing to additional milk protein yield. This may result in ammonia volatilization, nitrate leaching, and nitrous oxide emissions from manure during storage and after field application (James et al., 1999; Hristov et al., 2019). Moreover, increasing dietary CP reduces NUE (Colmenero and Broderick, 2006) and may affect income over feed cost (Wu et al., 2019) depending on production responses and feed costs. Therefore, there has been considerable interest in optimizing dietary CP level to maintain cow productive

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potential while minimizing the risk of environmental-N losses and reducing feed costs.

Dietary CP has historically been overfed relative to recommendations in US dairy herds (Jonker et al., 2002). Several dose response studies have evaluated the effect of dietary CP on cow performance. Reducing dietary CP concentrations to 16.5 and 15.5% for cows in early lactation (Colmenero and Broderick, 2006) and late lactation (Barros et al., 2017), respectively, did not have a detrimental effect on milk yield (**MY**), but further reductions resulted in reduced MY. Moreover, the NRC (2001) underestimation of MP-allowable milk when MP balance is negative (Lee et al., 2012) suggested that actual milk production loss would be less than losses predicted by the NRC (2001). For example, late lactation cows produced 8.0, 6.4, 3.6, and 2.6 kg/d more milk than NRC-predicted when fed diets with 11.8, 13.1, 14.4, and 16.1% CP, respectively (Barros et al., 2017). This underestimation could be because the NRC (2001) database was built using studies with cows in early to mid-lactation fed 17.1 ± 2.6% dietary CP. In a study performed over the duration of a lactation, feeding 17.3% CP from 1 to 150 DIM followed by a reduction to 14.4% CP after 151 DIM did not affect MY compared with continuous feeding of 17.3% CP over the lactation (Law et al., 2009). Feeding 17.4% CP from 1 to 119 DIM followed by a reduction to 16.0% CP after 120 DIM did not affect MY compared with feeding 17.9% CP over the lactation (Wu and Satter, 2000). These studies suggest there is an opportunity to reduce CP later in lactation without affecting cow performance. In addition, the results of a comprehensive meta-analysis suggested that responses to dietary CP concentration may differ with advancing DIM because including DIM in the statistical model improved model fit for MY (Hristov et al., 2004), However, Burgos et al. (2007) found no significant interaction between dietary CP concentration and stage of lactation on MY when dietary CP ranged from 15.1 to 20.7% and DIM ranged from 137 to 234 d during the course of the experiment.

Although many studies have evaluated the effects of changing dietary CP concentration, most studies either attempt to use cows at one stage of lactation or report pooled responses to dietary CP concentration changes across stages of lactation. To accurately feed dietary CP to cows across stages of lactation, it is imperative to know the optimal range of dietary CP that sustains cow performance throughout lactation, but few studies are available that simultaneously address each of these factors. Therefore, our primary objective was to evaluate the effect of 4 levels of dietary CP concentration on production performance, feed efficiency (**FE**), and NUE when fed to cows grouped as early, mid-early, mid-late, and late lactation. Our second objective was to determine the range of dietary CP concentrations at which the production responses are not negatively affected across different DIM. Based on the results of previous feeding studies, our main hypothesis was that there is no cow performance improvement when feeding dietary CP greater than ~16.5% across lactation stages. Our second hypothesis was that there is an interaction by which cow production responses to dietary CP decreases with advancing DIM.

MATERIALS AND METHODS

Cows, Experimental Design, and Dietary Treatments

This study was conducted with 64 multiparous Holstein cows housed in tiestalls at the Dairy Forage Research Center dairy farm in Prairie du Sac, Wisconsin. The experimental procedures for the use and care of animals were approved by the University of Wisconsin Institutional Animal Care and Use Committee (protocol no. A005043-R01-A03). Four stages of lactation groups were created by sorting cows from the lowest to the highest DIM and separating them into 4 groups of 16 cows: early (beginning DIM and parity mean ± SD, respectively; 86 ± 14.9; 2.3 ± 0.49), mid-early (119 ± 10.0; 2.6 ± 0.91), mid-late (167 ± 22.2; 2.7 ± 1.08), and late (239 ± 11.1; 2.5 ± 0.82). Within stage of lactation, cows were randomly assigned to 1 of 4 dietary treatments formulated for 13.5, 15.0, 16.5, or 18.0% CP (DM basis) by exchanging solvent and expeller soybean meal for soyhulls and dried ground corn. The study was conducted from September 29, 2019, to December 7, 2019, and consisted of a 10-wk experimental period including a 14-d covariate period during which cows were fed the common herd diet followed by 56 d of a dietary treatment period.

Feeding, Feed Sampling, and Chemical Analysis

Cows were individually fed TMR once a day at 1000 h with the amount offered allowing for 5 to 10% refusals. Offered TMR was adjusted weekly for changes in forage DM concentration. Samples of forages and high-moisture corn were collected daily for 7 d and composited by week on a wet basis whereas orts samples were collected daily and composited by dietary treatment and sampling week. These samples were frozen at −20°C. Samples of corn, soybean hulls, soybean meal, expellers soybean meal, roasted soybeans, and canola meal were collected once per week and stored at room temperature. After thawing the samples at room temperature, all feed ingredients and orts samples were dried at 55°C for 48 h and ground in a Wiley mill (Thomas Scientific) to pass a 1-mm screen. To determine the chemical composition of dietary treatments, feed ingredients were dried at 55°C for 48 h and analyzed by Dairyland Laboratories Inc. for absolute DM by drying at 105°C for 3 h, N by combustion method (method 990.03, AOAC International, 2006), ADF and lignin (method 973.18, AOAC International, 1996), NDF, treated amylase and sodium sulfate and ash-corrected NDF (method 2002.04, AOAC International, 2005), crude fat using diethyl ether extract as solvent (method 920.39, AOAC International, 1996), and ash at 550°C for 2 h to calculate OM (method 942.05, AOAC International, 1996). The DM and chemical composition of the TMR offered was calculated using individual feed ingredients and the proportion of each feed ingredient mixed into the TMR. Dry matter intake was calculated as TMR offered minus TMR refused multiplied by their respective DM concentration. Predictions for MP, RDP, and RUP were estimated from the NRC (2001) based on actual performance (DMI, MY, and milk composition), BW, average DIM during the experiment and the measured chemical composition of the actual ingredients.

Milk Sample Collection, BW, and BCS

Cows were milked 3 times daily at approximately 0600, 1300, and 2100 h. Milk yield was recorded daily, and samples were collected from 6 consecutive milkings every week except for wk 2 (last week of the covariate period), 6, and 10 during which samples were collected from 12 consecutive milking. Samples were preserved using bronopol and analyzed for milk true protein, fat, lactose, and MUN by infrared using a Foss FT6000 (Foss Electric; Agsource Milk Analysis Laboratory). Milk compositional analysis was the only time during the experiment that blinding to treatment occurred. Chemical analytes were weighted by the partial yield of the corresponding milking to calculate daily milk composition. Daily MY was obtained by summing the recorded amount at each milking, and daily milk component yields were obtained by multiplying the weighted milk composition by the daily MY. Fat- and protein-corrected milk (**FPCM**) was calculated according to IDF (2015), which standardizes MY for 4% fat and 3.3% true protein concentration, and milk NE_L was calculated according to NRC (2001). Feed efficiency was calculated by dividing kg of MY, FPCM, and Mcal of milk NE_L by kg of DMI. The NUE was calculated as 100 × milk true protein-N (kg) divided by N intake (kg). Cow BW was measured upon exit from the parlor on 2 consecutive days every week except for wk 2, 6, and 10 during which it was measured in 4 consecutive days. Change in BW for each cow was calculated as the slope of a linear regression of BW on experimental day for the duration of the experiment. Body condition scores were determined during wk 2, 6, and 10 by 3 different trained individuals scoring from 1 to 5 (Wildman et al., 1982).

Statistical Analysis

The experimental design and animal numbers were chosen based on a power calculation for $\alpha = 0.05$ and $\beta < 0.25$ using variance components estimates from similar research, a maximum effect size response equal to the SD for milk protein production, and to balance the duration of the experiment with the length of lactation. During the experiment, 1 cow in early lactation assigned to the 18.3% CP diet and 1 cow in mid-early lactation assigned to 13.6% CP diet were removed from the study due to acute clinical mastitis with associated sharp drops in milk production and DMI leaving 62 cows in the analysis. All the responses were averaged and statistically analyzed by week. Data were analyzed as a complete randomized design using a mixed-effect model with stage of lactation and concentration of dietary CP as categorical variables arranged in a 4 × 4 factorial. The covariate was calculated as the residual of the regression line fitted for the response variable and cow DIM, which accounts for variation associated with cow independent of the magnitude of the response variable (Ceyhan and Goad, 2009). Raw data were analyzed for outliers by visual observation of the response versus time plots. Additionally, we calculated the percent change in the response over time, carefully examined the changes, and discarded if values were not reasonable. The discarded values were then imputed by the average of the 3 previous and 3 subsequent values. The DMI, MY, milk chemical composition, milk component yield, BCS, BW, and BW change were analyzed using week as a repeated measure. After evaluating first-order autoregressive [AR(1)] and heterogeneous first-order autoregressive [AR(1)] variance-covariance structures, we selected AR(1) based on the smallest AIC to run the following statistical model:

$$Y_{ijkl} = \mu + Cov_i + L_j + D_k + W_l + LD_{jk} + LW_{jl} + DW_{jl} + LDW_{jkl} + c_{i:jk} + e_{ijkl},$$

where Y_{ijkl} is the response variable ($ijkl = 496$); i = cows (i = 62), j = stage of lactation (j = 1–4), k = dietary CP concentration (k = 1–4); l = experimental week (l = 1–8); μ = overall mean; Cov_i = residual covariate for cow i; L_j = fixed effect of stage of lactation j; D_j = fixed effect of diet k; W_l = fixed effect of experimental week l; DL_{jk} = is the fixed effect of the interaction between diet and stage of lactation; DW_{jl} = fixed effect of the interaction between diet and experimental week; LW_{kl}

= fixed effect of the interaction between stage of lactation and experimental week; LDW_{jkl} = fixed effect of the 3-way interaction; $c_{i:jk}$ = random effect of cow i within dietary treatment j and stage of lactation k; and e_{ijkl} = random residual error.

Statistical and residual analysis (studentized residuals to check for outliers) was conducted using the Mixed procedure of SAS version 9.4 (SAS Institute Inc.). We conducted linear, quadratic, and cubic contrasts of the main effects of stage of lactation, dietary treatment, and their linear interaction. Least squares means of production responses for significant interaction of stage of lactation and dietary treatment were used to visualize the response surface using the plotly package of R Studio Version 1.3.1056.

To achieve our second objective, a multiple regression predictive model with continuous independent variables of DIM and dietary CP concentration was used to describe the continuous response surface and to determine the range of dietary CP at which the production responses were not negatively affected (see details below). For this analysis, the DIM values corresponded to the day that the milk samples were obtained and dietary CP concentration was based on the samples collected and analyzed weekly. The model included linear, quadratic, and cubic slopes for DIM and dietary CP concentration, linear and quadratic interactions between DIM and dietary CP concentration, and the random effect of cow. The model was as follows:

$$Y_{ijk} = B_o + B_cCov + B_1DIM_j + B_2DIM^2{}_j + B_3DIM^3{}_j + B_4CP_k + B_5CP^2{}_k + B_6CP^3{}_k + B_7(\mathrm{CP} \times DIM)_{kj} + B_8(CP^2 \times DIM^2)_{kj} + b_{i,0} + e_{ijk},$$

where Y_{ijk} = dependent, continuous response variable ($ijk = 496$); i = individual cows ($i = 62$), j = continuous DIM (ranging from 79 to 323 DIM), k = continuous dietary CP concentration (ranging from 13.6 to 18.3% CP); B_o = overall fixed effect intercept; B_cCov = fixed effect of the residual covariate per cow; DIM_j = DIM independent variable; CP_k = dietary CP concentration independent variable; B_1 = overall fixed effect linear slope parameter estimate for DIM; B_2 = overall fixed effect quadratic slope parameter estimate for DIM; B_3 = overall fixed effect cubic slope parameter estimate for DIM; B_4 = overall fixed effect linear slope parameter estimate for dietary CP; B_5 = overall fixed effect quadratic slope parameter estimate for dietary CP; B_6 = overall fixed effect cubic slope parameter estimate for dietary CP; B_7 = overall fixed effect linear slope parameter estimate for the linear interaction between DIM and dietary CP; B_8 = overall fixed effect quadratic slope parameter estimate for the quadratic interaction between DIM and dietary CP; $b_{i,0}$ = random effect of i on the intercept; e_{ijk} = residual error. Factors in the model were removed only if higher order interactions were not significant.

Data were centered and scaled to the average DIM and dietary CP concentration. This adjustment was performed so that the intercept of the predictive model was within the data range and not an extrapolation. Using the parameter estimates of the predictive model, the dietary CP at the maximum predicted performance responses were calculated by setting the first derivative of the function to 0 and solving for CP using the quadratic formula across the range of DIM in this study, where CP of the maximum is restricted to be within the range of dietary CP concentration in this study. The maximum predicted performance responses were calculated by entering dietary CP at the maximum performance for each DIM to the predictive model. Ninety-five percent confidence interval (CI) of the maximum predicted performance responses were calculated to determine the lower limit of the predicted maximum performance (lowest level of production that did not differ from the predicted maximum) and the range of dietary CP to achieve the response (Supplemental Figure S1; https://doi.org/10.5281/zenodo.6958763; Letelier et al., 2022). The predictive model was fit using the NLMIXED procedure of SAS version 9.4 (SAS Institute Inc.). Results are discussed for DMI and milk NE_L output as an aggregated response of MY and milk component yield. Graphical figures were created using Microsoft Excel. For all analyses, $P \leq 0.05$ was considered significant and $0.05 < P \leq 0.10$ values were considered a tendency.

RESULTS AND DISCUSSION

Cow Characteristics and Chemical Composition of the Diet

Summary statistics for performance, milk component yield, and efficiencies of remaining cows during the covariate period are in Table 1. Figure 1 depicts DIM of cows with advancing experimental days. The average ± SD DIM of cows during the treatment period was 128 ± 14.9, 162 ± 10.0, 210 ± 22.3, and 282 ± 11.2 in early, mid-early, mid-late, and late lactation, respectively. In this discussion, we will refer to the DIM groups by these categorical descriptors of lactation stage although DIM is changing continuously throughout the duration of the study and these descriptions of DIM may be subjectively different for different users.

Table 1. Cow characteristics at each stage of lactation during the covariate period[1]

Item	Early (n = 15)		Mid-early (n = 15)		Mid-late (n = 16)		Late (n = 16)	
	Average	SD	Average	SD	Average	SD	Average	SD
DIM[2]	86	14.9	119	10.0	167	22.2	239	11.1
Parity	2.3	0.49	2.6	0.91	2.7	1.08	2.5	0.82
BW	635	56.1	664	50.6	655	54.6	702	62.1
DMI, kg/d	28.9	2.70	30.4	3.34	29.1	2.69	28.3	2.81
N intake, g/d	794	7.4	837	9.2	800	7.4	779	7.7
Milk, kg/d	49.3	4.84	49.7	5.36	44.8	6.15	37.4	4.35
FPCM,[3] kg/d	45.7	5.12	47.0	4.31	43.6	4.93	37.7	4.48
NE_L,[4] Mcal/d	34.1	3.88	35.2	3.21	32.6	3.75	28.0	3.41
Milk component yield, g/d								
True protein	1,399	115	1,449	140	1,366	142	1,202	137
Fat	1,834	287	1,900	214	1,779	245	1,548	218
Lactose	2,386	238	2,442	265	2,174	323	1,744	230
Milk composition								
True protein, %	2.85	0.155	2.93	0.182	3.07	0.210	3.22	0.162
Fat, %	3.72	0.430	3.85	0.427	4.00	0.509	4.15	0.447
Lactose, %	4.84	0.132	4.92	0.125	4.84	0.134	4.74	0.168
MUN, mg/dL	9.29	1.172	9.15	1.260	9.54	1.251	9.72	1.253
Feed efficiency								
Milk/DMI	1.71	0.150	1.64	0.138	1.55	0.197	1.33	0.151
FPCM/DMI	1.59	0.158	1.55	0.124	1.51	0.172	1.34	0.155
NE_L/DMI	1.19	0.121	1.16	0.095	1.13	0.130	0.99	0.119
NUE,[5] %	27.7	2.11	27.3	2.46	26.9	2.94	24.3	2.63

[1]All cows were fed the same diet during the 2-wk covariate period. Data are for cows that completed the experiment.

[2]Days in milk on d 1 of the covariate period.

[3]Fat- and protein-corrected milk calculated according to IDF (2015).

[4]Milk net energy for lactation calculated according to NRC (2001).

[5]Nitrogen use efficiency = milk true protein-N (g/d):N intake (g/d) × 100.

Dietary ingredients and chemical composition of dietary treatments are in Table 2 and chemical composition of feed ingredients is in Table 3. Whereas MP, and more precisely metabolizable AA, is the required nutrient for dairy cattle, CP is an analytical entity that is not dependent on animal characteristics such as feed intake that may change throughout the course of lactation. For this reason, we altered dietary CP concentration when fed to cows across 4 DIM groups. Dietary CP ranged from 13.6 to 18.3%. The calculated RDP (NRC, 2001) ranged between 10.3 to 12.7% DM, and the RDP balance ranged between 221 to 948 g/d across dietary treatments, indicating that all the diets had an excess of ruminal available N. The RUP balance was negative for all, except for the 18.3% dietary treatments.

The ingredient exchange to obtain the desired range in dietary CP concentration resulted in a change of 3.9 percentage units in aNDF (decrease from 31.8 to 27.9% of dietary DM as dietary CP increased from 13.6 to 18.3%) and a decrease of 3.3 units in starch (decrease from 26.8 to 23.5% of dietary DM as dietary CP increased from 13.6 to 18.3%). Changes to dietary CP concentration is impossible without changing other chemical component concentrations in a feed substitution study. As such, we cannot exclude the possibility that these other feed components (especially starch and NDF concentration changes) could be contributing to the responses observed from changes in CP concentration. However, for clarity we will discuss dietary changes with respect to the changes in dietary CP concentration. The NE_L balance predicted by the NRC (2001) was positive (>2.3 Mcal/d) for all dietary treatments, contrary to the MP balance, which was negative for all, except for the 18.3% dietary treatments (−444, −278, −109, and 173 g/d for 13.6, 15.2, 16.7, and 18.3% dietary CP treatment, respectively). Although the lowest 3 dietary CP treatments resulted in negative MP balance, the NRC (2001) is known to underpredict MP allowable milk with reducing dietary CP (Lee et al., 2012).

Performance Responses to Stage of Lactation and Dietary CP Concentration

The effect of stage of lactation and dietary CP concentration on BW, DMI, performance, milk composition, and efficiencies are shown in Table 4 and Supplemental Table S1 (https://doi.org/10.5281/zenodo.6958763; Letelier et al., 2022). The DIM discussed in the works cited in this discussion correspond to the calculated average DIM during the treatment period based on the reported initial DIM, duration of the covariate (if

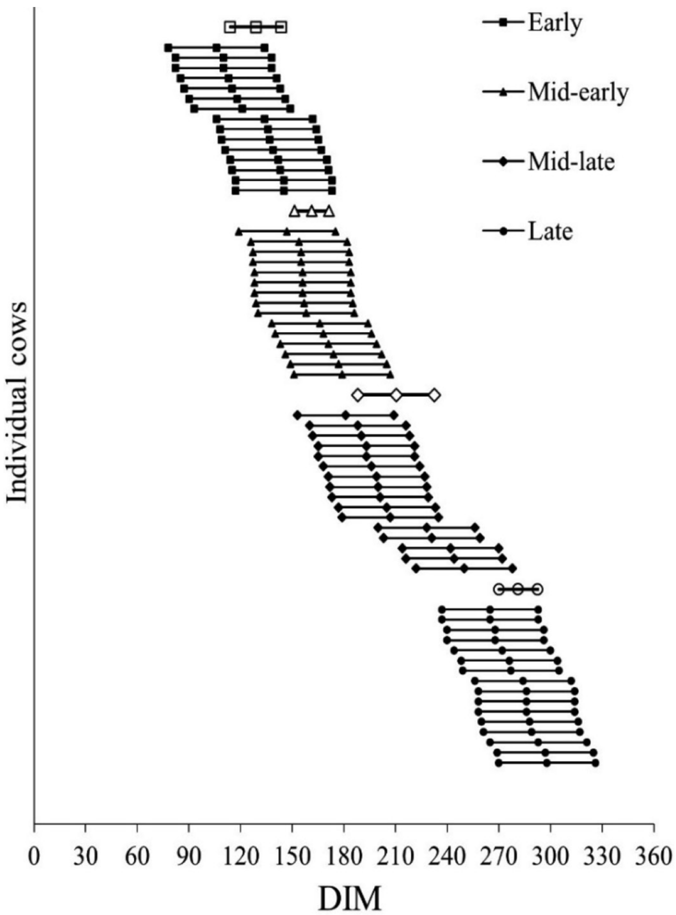

Figure 1. Days in milk of cows enrolled in the experiment starting at d 1 of the treatment period. Each line represents a cow (n = 62). Closed squares, closed triangles, closed diamonds, and closed circles represent cows in early (n = 15), mid-early (n = 15), mid-late (mid-late = 16), and late lactation (n = 16), respectively. The left, middle, and right symbols in the line represent the initial, average, and last DIM of each cow during the experiment, respectively. The open symbols represent the average ± SD of DIM of the group of cows in each stage of lactation.

applicable) and treatment period (where the average DIM during the treatment period in this study is 196 d). Body weight and BCS linearly increased by 77 kg and 0.35 units from early to late lactation, respectively. The rate of BW change tended to linearly increase 0.30 kg/d from early to late lactation. Additionally, a linear tendency for increased rates of BW gain were observed with increased CP concentration. Colmenero and Broderick (2006) evaluated 5 CP levels (between 13.5 to 19.4% CP) in an incomplete 5 × 5 Latin square experimental design using mid-early lactation cows (averaging 176 DIM), and reported an overall average of 0.57 kg/d of BW gain but no effect of dietary CP levels. Barros et al. (2017) evaluated 4 levels of dietary CP (between 11.8 and 16.2% CP) in a randomized complete block experimental design using late-lactation cows (averaging 287 DIM) and reported an average of 0.31 kg/d of BW gain and a quadratic effect of dietary CP level.

In this study, DMI response to dietary CP concentration depended on the stage of lactation (Table 4). Early, mid-early, and late lactation cows had the greatest DMI when fed diets with 16.7% CP (32.9, 31.3, and 29.1 kg/d, respectively), whereas mid-late lactation cows had the greatest DMI when fed diet with 18.3% CP (31.1 kg/d; Figure 2A). Similarly, we observed an interaction for FPCM and milk NE_L output between stage of lactation and dietary CP. When feeding the 13.6% CP diet, early lactation cows produced 7.0 Mcal/d more than cows in late lactation (30.4 vs. 23.4 Mcal/d), whereas when fed 18.3% CP diets early lactation cows produced 14.7 Mcal/d more than cows in late lactation (35.3 vs. 20.6 Mcal/d; Figure 2B). Moreover, cows in early lactation fed diets containing 16.7% CP produced 0.81 Mcal/d more milk NE_L compared with cows fed 18.3% CP diet, whereas cows in late lactation fed 16.7% CP diet produced 5.2 Mcal/d more milk NE_L compared with cows fed 18.3% CP diet. The larger drop of milk NE_L output in late compared with early lactation when cows were fed diets with 18.3% CP suggested that excess of dietary CP in later stages of lactation negatively affected milk NE_L output. Similarly, with cows averaging 175 DIM, Burgos et al. (2007) reported a numerical decrease of 1.6 kg MY/d when dietary CP increased from 16.6 to 18.6% CP, whereas Katongole and Yan (2020) reported a numerical decrease of 3.3 kg MY/d when dietary CP increased from 17.7 to 20.1% CP in cows averaging 187 DIM. However, this effect was not observed in mid-late lactation cows averaging 167 DIM in this study. A portion of the large drop in milk NE_L output could be due to the energy expended on excreting the excess of N consumed relative to ruminal N or metabolizable AA requirements, which has been estimated to reduce milk gross energy output by up to 68 kcal per g of excess fed N (Reed et al., 2017). In this study, feeding diets with 18.3% CP in late lactation resulted in 51 g/d of extra N compared with diets with 16.7% CP (831 vs. 780 g of N intake, respectively). This would result in 3.5 out of 5.3 Mcal/d (25.9–20.6 Mcal milk NE_L/d) milk gross energy expended for N excretion or 67.3% of the difference between these diets in late lactation cows.

As expected, MY, FPCM, and milk NE_L output linearly decreased from early to late lactation (−15.2 kg/d, −13.0 kg/d, and −9.9 Mcal/d, respectively) with a tendency toward a cubic response for FPCM and milk NE_L output (Table 4). Additionally, dietary CP concentration had a linear and quadratic effect on

Table 2. Ingredients and chemical composition of dietary treatments

Item	Covariate	Dietary CP % of the DM			
		13.6	15.2	16.7	18.3
Ingredient, % DM					
Alfalfa silage	34.3	26.5	26.4	26.4	26.4
Corn silage	27.5	34.7	34.6	34.6	34.6
High-moisture corn	6.5	10.6	10.6	10.6	10.6
Dry corn grain (rolled)	13.8	9.6	8.1	6.5	4.8
Soybean meal	—	—	3.0	6.1	9.2
Expeller soybean meal[1]	—	—	0.9	1.7	2.5
Soy hulls	—	8.4	6.1	3.8	1.5
Roasted soybean	6.8	—	—	—	—
Canola meal	8.9	3.7	3.7	3.7	3.7
Urea	—	0.4	0.4	0.4	0.4
Molasses	—	3.7	3.7	3.7	3.7
Mineral-vitamin premix[2]	2.3	2.6	2.5	2.5	2.5
Chemical composition[3]					
DM, % as-fed	60.7	53.1	53.2	53.7	53.2
CP	16.2	13.6	15.2	16.7	18.3
AD-ICP,[4] % CP	5.8	5.3	5.1	5.0	4.8
ND-ICP,[5] % CP	9.8	10.2	9.7	9.1	8.5
Soluble protein, % CP	42.7	83.9	83.7	46.8	46.6
ADF	23.6	23.5	22.5	21.6	20.6
aNDF[6]	32.5	33.5	32.2	30.9	29.6
aNDFom[7]	30.4	31.8	30.5	29.2	27.9
Lignin, % NDFom	13.8	8.4	8.4	8.4	8.4
Sugar	4.8	5.1	5.6	6.1	6.5
Starch	24.4	26.8	25.7	24.7	23.5
Ether extract	4.3	3.1	3.2	3.2	3.2
Ash	5.6	5.5	5.7	5.8	5.9
NRC (2001) prediction[8]					
NFC	—	46.0	45.7	45.3	44.8
NE_L, Mcal/kg	—	1.47	1.48	1.50	1.50
NE_L, Mcal/d	—				
Supplied	—	41.2	42.9	45.5	45.2
Required	—	39.0	39.9	42.4	40.9
Balance	—	2.3	3.0	3.2	4.4
CP					
RDP, % CP	—	75.3	72.4	70.7	69.4
RUP, % CP	—	24.7	27.6	29.3	30.6
RDP, % DM	—	10.3	11.0	11.8	12.7
RUP, % DM	—	3.3	4.2	4.9	5.6
MP, g/d					
Supplied	—	2,277	2,589	2,980	3,137
Required	—	2,721	2,867	3,089	2,963
Balance	—	−444	−278	−109	173
RDP, g/d					
Supplied	—	2,892	3,195	3,627	3,857
Required	—	2,672	2,757	2,909	2,857
Balance	—	221	437	718	948
RUP, g/d					
Supplied	—	929	1,207	1,533	1,721
Required	—	1,525	1,560	1,668	1,514
Balance	—	−596	−535	−134	207

[1]SoyPlus (Landus Cooperative).

[2]Containing (DM basis): 16.8% Ca, 4.64% Mg, 0.6% K, 15.8% Na, 7.1% Cl, 1.0% S, 45 mg/kg Co, 554 mg/kg Cu, 64 mg/kg I, 778 mg/kg Fe, 2,601 mg/kg Mn, 15.6 mg/kg Se, 2,808 mg/kg Zn, 311 kIU/kg vitamin A, 62 kIU/kg vitamin D, 1,453 IU/kg vitamin E, 523 mg/kg rumensin.

[3]Nutrients expressed as percentage of the DM unless stated otherwise and calculated using individual feed ingredients and the proportion of each feed ingredient mixed into the TMR.

[4]AD-ICP = acid detergent insoluble CP.

[5]ND-ICP = neutral detergent insoluble CP corrected with sodium sulfite.

[6]aNDF = NDF corrected with amylase and sodium sulfite.

[7]aNDFom = aNDF organic matter; corrected by ash concentration.

[8]NRC (2001) predictions were estimated based on actual performance (DMI, milk yield, and milk composition), BW, average DIM during the experiment and actual ingredients chemical composition.

Table 3. Chemical composition of dietary ingredients[1]

Chemical composition[2]	Alfalfa silage	Corn silage	High-moisture corn	Dry corn	Soybean meal	Expeller soybean meal	Soy hulls	Canola meal
DM, % as-is	40.1 (0.49)	33.0 (0.17)	71.9 (1.25)	83.0 (0.84)	85.4 (1.31)	86.2 (1.26)	86.8 (1.47)	83.7 (0.18)
CP	21.5 (0.21)	7.6 (0.06)	7.1 (0.25)	8.2 (0.39)	51.7 (0.41)	45.9 (0.73)	12.2 (0.46)	40.8 (0.40)
AD-ICP,[3] % CP	6.2 (0.26)	6.5 (0.80)	2.8 (0.50)	4.1 (1.10)	0.7 (0.04)	1.2 (0.16)	7.0 (0.50)	5.2 (0.17)
ND-ICP,[4] % CP	7.6 (0.28)	12.1 (0.40)	10.1 (0.26)	7.6 (1.74)	1.1 (0.02)	5.9 (0.57)	22.6 (4.46)	8.7 (0.88)
Soluble protein, % CP	64.6 (2.06)	57.8 (2.66)	22.3 (1.58)	16.3 (5.11)	23.7 (0.49)	13.2 (0.81)	35.1 (0.81)	14.5 (0.65)
ADF	35.2 (0.06)	24.9 (1.34)	2.3 (0.13)	2.5 (0.16)	6.1 (0.07)	9.4 (0.35)	51.4 (1.69)	20.1 (1.00)
aNDF[5]	39.9 (0.56)	42.2 (1.26)	7.4 (0.06)	7.0 (1.05)	9.1 (0.35)	15.3 (0.38)	70.5 (0.10)	27.1 (0.36)
aNDFom[6]	37.9 (0.78)	40.4 (1.32)	6.4 (0.30)	6.1 (0.49)	8.4 (0.01)	14.3 (0.43)	68.1 (0.20)	22.8 (0.91)
Lignin, % NDFom	18.9 (0.07)	3.6 (0.41)	1.2 (1.51)	3.4 (4.57)	3.9 (0.34)	2.6 (3.52)	2.9 (1.04)	38.5 (3.20)
Sugar	3.5 (0.28)	1.5 (0.21)	1.7 (0.19)	3.5 (0.91)	19.1 (0.88)	16.6 (0.01)	8.6 (0.13)	12.0 (0.76)
Starch	2.2 (0.08)	33.1(0.19)	73.2 (0.19)	71.4 (0.40)	1.6 (0.06)	1.4 (0.25)	0.7 (0.07)	1.3 (0.58)
Ether extract	3.8 (0.23)	3.4 (0.35)	2.6 (0.29)	3.0 (0.31)	2.6 (0.13)	6.4 (0.20)	2.4 (0.45)	4.9 (0.45)
Ash	10.3 (0.12)	3.8 (0.01)	1.5 (0.02)	1.5 (0.13)	7.1 (0.06)	6.6 (0.06)	4.9 (0.12)	8.1 (0.24)

[1]Composite samples by week for treatment wk 4 and 8 (n = 2); values in parentheses indicate standard deviation.

[2]Nutrients expressed as percentage of the DM unless stated otherwise.

[3]AD-ICP = acid detergent insoluble CP.

[4]ND-ICP = neutral detergent insoluble CP corrected with sodium sulfite.

[5]aNDF = NDF determined with amylase and sodium sulfite.

[6]aNDFom = aNDF organic matter; corrected for ash concentration.

MY, FPCM, and milk NE_L output, a cubic effect on FPCM and milk NE_L output, and cubic tendency on MY. Quadratic responses of milk production have been reported by other authors. For example, Colmenero and Broderick (2006) using cows in mid-early lactation (176 DIM) found a quadratic effect of dietary CP on 3.5% FCM where the greatest production was at 16.7% CP. Similarly, using cows in late lactation (287 DIM), Barros et al. (2017) found a quadratic effect of dietary CP on FPCM with a plateau response at 15.5% CP. Finally, Law et al. (2009) found no difference in MY when dietary CP was decreased to 14.4 from 17.3% CP at 151 DIM until 305 DIM, but cows had greater MY (+3.6 kg/d) when dietary CP concentration was 17.3% compared with 14.4% from 1 to 150 DIM.

We observed an interaction between stage of lactation and dietary CP concentration for yields of milk protein, fat, and lactose (Table 4). When fed the 13.6% CP diet, early lactation cows produced 199, 344, and 705 g/d more milk protein, fat, and lactose than cows in late lactation, respectively (Figure 2C, 2D and 2E). In contrast, when fed the 18.3% CP diet, early lactation cows produced 524, 812, and 1,081 g/d more milk protein, fat, and lactose than cows in late lactation, respectively. This result indicates that the magnitude of the difference in milk component yields from early to late lactation was smaller when cows were fed 13.6% compared with 18.3% CP diet. Similarly, Law et al. (2009) reported that when cows were fed a 11.4% CP diet, early lactation cows (75 DIM) produced 40 g/d milk protein more than cows in late lactation (228 DIM), however when fed a 17.3% CP diet, early lactation cows produced 120 g/d more milk protein than cows in late lactation. Moreover, cows in early lactation fed the 16.7% CP diet secreted 38 g/d more milk protein compared with cows fed the 18.3% CP diet, whereas cows in late lactation fed the 16.7% CP diet secreted 198 g/d more milk protein compared with cows fed the 18.3% CP diet. In this study, the greater magnitude of the drop in milk protein production in late lactation compared with early lactation when fed 18.3 versus 13.6% CP could be due to could be due to the decreasing ability of the cows to respond to additional dietary CP with increasing DIM. During early lactation, growth hormone concentration in plasma increases as a physiological adaptation to maintain lactation and stimulate glucose and acetate partitioning from peripheral tissues to the mammary gland (Baumgard et al., 2017). Moreover, GH has been shown to activate protein synthesis in the mammary gland through the mechanistic target rapamycin pathway (Hayashi and Proud, 2007; Sciascia et al., 2013), potentially explaining why cows in early lactation had greater responsiveness to AA availability compared with late lactation cows. Furthermore, the total number of active secretory cells has been shown to progressively decrease after parturition reducing the sensitivity of the mammary gland to nutrients supplied (Hanigan et al., 2007). Pregnancy status could also partially explain the large drop in milk protein yield in late lactation cows fed 18.3% CP, which was numerically more advanced (184 ± 23.8 d pregnant) compared with cows fed 13.6, 15.2 and 16.7% CP (145 ± 61.4, 169 ± 29.4, and 149 ± 39.6, respectively). After 100 d of pregnancy, the fetal weight increases exponentially, increasing competition between nutrients used for lactation and fetal growth (Bauman and Currie,

Table 4. Effect of stage of lactation and dietary CP on cow performance and feed efficiency

Item	Stage of lactation[1]				CP, % of DM				SEM[2]	*P*-value						
										Lactation			CP			Lactation × CP
	E	ME	ML	L	13.6	15.2	16.7	18.3		Lin[3]	Quad[4]	Cub[5]	Lin	Quad	Cub	
BW, kg	653	660	693	730	686	679	684	687	9	<0.01	0.25	0.14	0.63	0.28	0.43	0.75
BW change,[6] kg/d	0.41	0.21	0.32	0.71	0.28	0.40	0.49	0.50	0.208	0.10	0.94	0.53	0.09	0.53	0.56	0.14
BCS	3.07	3.12	3.27	3.42	3.24	3.22	3.22	3.21	0.077	<0.01	0.91	0.52	0.65	0.99	0.78	0.67
DMI, kg/d	30.4	29.7	29.5	28.4	28.0	29.0	30.8	30.1	0.60	<0.01	0.83	0.36	<0.01	<0.01	0.01	0.03
N intake, g/d	781	759	757	727	612	706	824	882	15	<0.01	0.92	0.18	<0.01	0.02	0.01	0.01
Milk yield, kg/d	45.4	41.7	38.7	30.2	36.5	38.3	41.6	39.5	1.57	<0.01	0.23	0.18	<0.01	0.02	0.06	0.12
FPCM,[7] kg/d	44.6	41.2	39.4	31.6	37.1	38.4	41.8	39.5	1.45	<0.01	0.15	0.07	<0.01	0.02	0.02	0.03
Milk NE_L,[8] Mcal/d	33.2	30.6	29.2	23.3	27.5	28.5	31.0	29.3	1.09	<0.01	0.15	0.07	<0.01	0.02	0.02	0.03
Milk composition																
True protein, %	3.07	3.14	3.29	3.47	3.20	3.25	3.26	3.26	0.056	<0.01	0.92	0.51	0.16	0.35	0.77	0.15
Fat, %	4.09	4.03	4.22	4.39	4.29	4.17	4.12	4.16	0.099	<0.01	0.23	0.13	0.06	0.11	0.89	0.35
Lactose, %	4.80	4.76	4.71	4.57	4.74	4.70	4.73	4.67	0.046	<0.01	0.35	0.41	0.11	0.80	0.12	0.17
MUN, mg/dL	11.4	11.1	11.0	12.0	8.0	9.8	12.4	15.3	0.359	0.01	<0.01	0.55	<0.01	<0.01	0.69	<0.01
Milk component yield, g/d																
True protein	1,385	1,302	1,263	1,038	1,152	1,229	1,342	1,266	51	<0.01	0.10	0.12	<0.01	<0.01	0.05	<0.01
Fat	1,835	1,682	1,622	1,309	1,544	1,576	1,714	1,616	62	<0.01	0.22	0.04	0.02	0.04	0.02	<0.01
Lactose	2,183	1,987	1,825	1,387	1,737	1,808	1,972	1,863	78	<0.01	0.25	0.19	<0.01	0.03	0.04	0.03
Feed efficiency																
MY/DMI[9]	1.49	1.42	1.31	1.06	1.30	1.32	1.35	1.31	0.051	<0.01	0.06	0.73	0.60	0.28	0.41	0.22
FPCM/DMI[10]	1.47	1.40	1.34	1.11	1.32	1.33	1.36	1.31	0.047	<0.01	0.04	0.25	0.94	0.24	0.30	0.03
NE_L/DMI[11]	1.09	1.04	0.99	0.82	0.98	0.98	1.01	0.97	0.035	<0.01	0.05	0.26	0.99	0.26	0.27	0.04
NUE,[12] %	28.2	27.3	26.7	22.8	29.7	27.3	25.6	22.5	0.947	<0.01	0.03	0.27	<0.01	0.48	0.30	0.29

[1]Average ± SD during the treatment period: E = early (128 ± 14.9 DIM); ME = mid-early (162 ± 10.0 DIM); ML = mid-late (210 ± 22.3 DIM); L = late (282 ± 11.2 DIM).
[2]SEM of the interaction of stage of lactation and dietary CP.
[3]Linear effect.
[4]Quadratic effect.
[5]Cubic effect.
[6]Calculated as the slope of a linear regression of BW on experimental day for the duration of the experiment.
[7]Fat- and protein-corrected milk calculated according to IDF (2015).
[8]NE_L calculated according to NRC (2001).
[9]Milk yield (kg/d): DMI (kg/d).
[10]FPCM (kg/d): DMI (kg/d).
[11]Milk net energy of lactation (Mcal/d): DMI (kg/d).
[12]Milk true protein-N:N intake × 100.

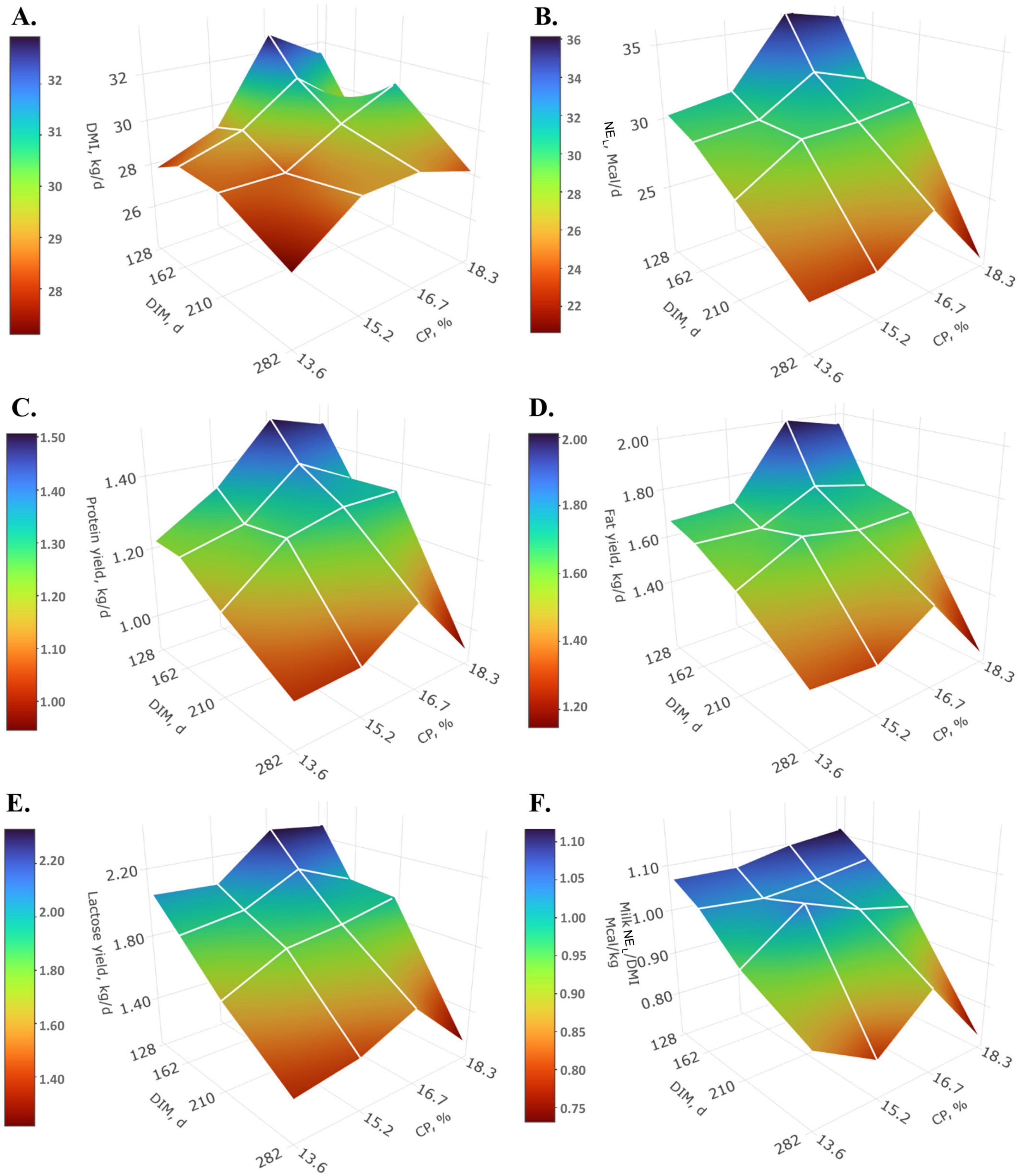

Figure 2. Dry matter intake (A), milk energy for lactation output (NE_L; B), milk protein yield (C), milk fat yield (D), milk lactose yield (E), and feed efficiency (milk NE_L/DMI; F) in response to DIM and level of dietary CP (%DM). Each data point in the surface is the LSM of the interaction between DIM (average during the experimental weeks) and dietary CP concentration.

1980). Distinguishing among these possible mechanisms requires further experimentation in late lactation cows.

Milk protein, fat, and lactose yield decreased linearly by 347, 526, and 796 g/d, respectively, from early to late lactation (Table 4). Additionally, stage of lactation had a cubic effect on milk fat yield, but not on other milk component yields. This response may be due to changes in fat formation pathways affecting milk fat composition differently across stages of lactation as has been shown previously (Stoop et al., 2009). The literature is inconsistent regarding fat yield responses to dietary CP. Similar to our finding, Broderick (2003) found a quadratic effect of dietary CP on fat yield, with the greatest production at 16.7%. However, Barros et al. (2017) found significant linear responses and only a tendency for quadratic responses of milk fat when dietary CP changed between 11.8 and 16.2% CP. In contrast, no milk fat yield response to changing dietary CP was reported in Colmenero and Broderick (2006). Dietary CP concentration had linear, quadratic, and cubic effects on milk component yield. Previous studies have shown no response of milk protein yield to dietary CP above 18.5% in early lactation (80 DIM; Cunningham et al., 1996), 16.5% in mid-early lactation (176 DIM; Colmenero and Broderick, 2006), 16.7% in mid-early lactation (186 DIM; Broderick, 2003), 16.0% in mid-late lactation (214 DIM; Wu and Satter, 2000), and 16.2% in late lactation (287 DIM; Barros et al., 2017). However, the responses of milk protein yield to increasing dietary CP concentration have also been variable depending on the forage components fed to cows in mid-early lactation (150 DIM; Groff and Wu, 2005). In that study, maximum milk protein yield occurred at 18.75, 16.25, and 17.50% CP for alfalfa silage to corn silage ratios of 75:25, 50:50, and 25:75, respectively. Diets fed to cows in our study had greater concentrations of corn silage and total dietary forage than any of the diets fed in Groff and Wu (2005), however the alfalfa to corn silage ratio of approximately 43:57 in the current study was between the diets that resulted in maximum milk protein yields where CP concentration was 16.25 and 17.5%. It is important to note, with this and other responses, that the effects of forage to concentrate ratio or the type of carbohydrates used to balance for changes in dietary CP could also be affecting the magnitude of the production response, but we were unable to separate these dietary factors in this study.

Burgos et al. (2007) evaluated 3 stages of lactation (137, 189, and 235 DIM) and 4 levels of dietary CP (15.1, 16.6, 18.6, and 20.7% CP). Contrary to our results, these authors found no interaction between stage of lactation and dietary CP for milk production or components responses. These discrepancies might be due to the source of dietary N. Burgos et al. (2007) replaced soyhulls with urea to increase CP concentration across dietary treatments, whereas in our study, dietary CP was increased with soybean meal sources. Urea is a highly soluble source of ruminal N which, if the N is not used by ruminal microbes, accumulates as NH_3-N in the rumen, is absorbed through the ruminal wall into portal circulation, undergoes ureagenesis in the liver, and is lost to the cow as urinary urea-N (Broderick et al., 1993). In contrast, using soybean protein provides a source of true protein-N that may be used by the ruminal microbes or absorbed as a source of RUP. Another possible explanation may be associated with the experimental design. Contrary to our continuous, completely randomized experimental design, the study of Burgos et al. (2007) was a split-plot Latin square design with stage of lactation as main plots and dietary CP concentration as subplots. Zanton (2019) found that the statistical inferences related to the effects of changes in dietary CP between continuous and change over designs were different for milk fat and protein percentage and milk NE_L output. Additionally, the 6-d adaptation period used in Burgos et al. (2007) may have been insufficient time for the ruminal microbial community to adapt to dietary changes, which has been estimated to be 10 d (Weimer et al., 2017).

We observed an interaction between stage of lactation and dietary CP for FE calculated as FPCM/DMI and NE_L/DMI (Table 4; Figure 2F), but not for MY/DMI. Stage of lactation had a linear and quadratic effect on FE, decreasing 0.43, 0.36, and 0.27 units from early to late lactation for MY/DMI, FPCM/DMI, and NE_L/DMI, respectively (Table 4), however dietary CP concentration did not independently affect any of the reported measures of FE. An interaction between DIM and CP level was also detected for MUN, which primarily resulted from increased MUN concentrations for cows in late lactation fed increasing levels of CP. Specifically, cows in late lactation fed the 18.3% CP diet had greater MUN concentration compared with cows in other stages of lactation fed that diet. However, the major factor affecting MUN was diet with concentration increasing from 8.0 mg/dL at 13.6% CP to 15.3 mg/dL at 18.3% CP. In contrast, although significant, DIM main effects on MUN were lesser than dietary CP concentration resulting in MUN concentration changes of less than or equal to 1 mg/dL on average. Nitrogen use efficiency exhibited a linear and quadratic decline from 28.2 to 22.8% for early to late lactation, and a linear decline from 29.7 to 22.5% as dietary CP increased from 13.6 to 18.3%, which was not affected by a DIM by CP interaction.

Response Curve to Dietary CP and DIM

Parameter estimates of the multiple regression models for DMI, milk NE_L output, FPCM, protein yield, and fat yield are in Table 5. The quadratic interaction between dietary CP concentration and DIM (DIM × DIM × CP × CP) was significant for most of these response variables, and thus cubic, quadratic, linear, and main effects were retained in the prediction models. In contrast, the quadratic and cubic main effect of dietary CP on measures of FE were not significant thus, a predicted maximum response did not apply within the range of dietary CP of our study. Figure 3 illustrates the sigmoidal response curves for DMI and milk NE_L output within the range of dietary CP concentration and DIM included in our study. For both variables, increasing levels of dietary CP resulted in a predicted increase in the dependent response variables, but was followed by a substantial decline in the upper range of dietary CP. The interactions between DIM and CP concentration resulted in an upward shift (from DIM 120 to 180) followed by a downward shift (from DIM 180 to 270) in the concentration of dietary CP associated with the predicted maxima in DMI and milk NE_L output. Notably, the lower boundaries of the range in dietary CP concentration for which these response variables did not differ from their maxima followed the same pattern, but the distance between the predicted maxima and the lower boundary of the CI widened with advancing DIM categories (Supplemental Table S2; https://doi.org/10.5281/zenodo.6958763; Letelier et al., 2022). Between DIM 120 and 220 the predicted dietary CP associated with maximal DMI was either 17.4 or 17.5%, and the lower boundary of the range for which DMI remain within the confidence limit was either 16.3 or 16.4%. However, as DIM increased above 220, the predicted dietary CP associated with the maximal DMI began to decline, as well as the lower boundary of the CI. A similar pattern was observed for NE_L with predicted dietary CP associated with maximal milk NE_L output and lower boundary of the CI being essentially 0.1 percentage unit lower than for DMI. According to our regression analysis, at 120 DIM, the range of dietary CP concentration that is predicted to not differ from the maximum was from 16.3 to 18.5% for DMI and from 16.2 to 18.4% for milk NE_L output, whereas the predicted maximum occurred at 17.4 and 17.3% CP, respectively. These levels of CP are similar to those determined with regression analyses conducted by Colmenero and Broderick (2006), which included a linear and quadratic but no cubic effect and predicted maximum MY when CP was 16.7% and maximum milk protein yield when CP was 17.1% for cows at 176 DIM on average. Additionally, in a study performed over the duration of a lactation, Wu and Satter (2000) recommended to feed diets containing a minimum of 17.5% CP in early lactation and not below 16.0% after mid lactation to sustain MY.

Furthermore, from our regression analysis, at 270 DIM the range of dietary CP that is predicted not to differ from the maximum was from 15.7 to 18.4% for DMI and from 15.5 to 18.3% for milk NE_L output, whereas the predicted maximum occurred at 17.1 and 16.9% CP, respectively. These results agreed with those of Barros et al. (2017) suggesting that for late lactation cows FPCM did not decline from its maxima until dietary CP was decreased below 15.5%. However, in their study the DMI response to dietary CP ranging from 11.8 to 16.2% was linear.

Thus, our results suggest a diminishing return to dietary CP concentration with advancing stage of lactation because in earlier stages of lactation, increasing dietary CP from 16.3% to 17.4% only marginally improved DMI and milk NE_L output whereas in later lactation the range of marginal improvement occurred at dietary CP from 15.7 to 17.1%. Feeding 120-DIM cows at the lower boundary of the CI (Supplemental Table S2) means that cows could achieve 97.2 and 96.4% of their predicted maxima for DMI and milk NE_L output, whereas reducing dietary CP by 1.1 percentage unit (essentially from 17.4 to 16.3%). Similarly, feeding 270-DIM cows at the lower boundary of the CI means that cows could achieve 97.3 and 95.0% of their predicted maxima for DMI and milk NE_L output, whereas reducing dietary CP by 1.4 percentage unit (essentially from 17.0 to 15.6%). Careful interpretation of these percentages is warranted as they are within the margin of model prediction error. Furthermore, the lowest level of dietary CP concentration that maintains performance within the predicted maxima may also vary with dietary formulation strategies and in particular the simultaneous optimization of the supply and composition of metabolizable energy and MP. In commercial settings, the actual level of dietary CP fed to cows at different stages of lactation varies depending on many factors that were outside the scope of this study. Nevertheless, the predictive equations in Table 5 may be used as a guide to help on-farm decision-making as we inferred from our findings that there is a range over which dietary CP could be reduced with limited risk for negative effects on cow productive performance. Finally, our analysis suggests that overfeeding dietary CP may negatively affect production responses, especially in late lactation.

Table 5. Parameter estimates of multiple regression predictive model with continuous independent variables of DIM and dietary CP concentration on performance measures of lactating cows

Variable	Intercept	Standardized DIM[1] Lin[3]	Standardized DIM[1] Quad[4]	Standardized DIM[1] Cub[5]	Standardized CP[2] Lin	Standardized CP[2] Quad	Standardized CP[2] Cub	DIM × CP	DIM × DIM × CP × CP	RMSE[6]	R^2
DMI, kg/d	29.5	−5.77E-02	3.28E-03	−1.11E-03	1.28	2.27E-02	−1.49E-01	−3.82E-02	−2.42E-03	1.60	72.7
SE	3.10E-01	4.05E-02	4.61E-03	4.17E-04	3.12E-01	8.88E-02	6.12E-02	1.31E-02	1.20E-03		
P-value	<0.01	0.16	0.48	0.01	<0.01	0.80	0.02	<0.01	0.04		
NE_L,[7] Mcal/d	29.3	−5.08E-01	−6.93E-03	−1.74E-03	1.87	8.75E-02	−2.76E-01	−8.18E-02	−3.90E-03	2.27	85.3
SE	4.85E-01	6.12E-02	6.87E-03	6.06E-04	5.09E-01	1.40E-01	9.97E-02	2.05E-02	1.77E-03		
P-value	<0.01	<0.01	0.31	<0.01	<0.01	0.53	0.01	<0.01	0.03		
FPCM,[8] kg/d	39.6	−6.60E-01	−9.08E-03	−2.38E-03	2.48	1.01E-01	−3.58E-01	−1.12E-01	−5.22E-03	3.04	85.0
SE	6.44E-01	8.14E-02	9.16E-03	8.09E-04	6.75E-01	1.86E-01	1.32E-01	2.72E-02	2.36E-03		
P-value	<0.01	<0.01	0.32	<0.01	<0.01	0.59	0.01	<0.01	0.03		
Milk component yield, g/d											
Protein	1,287	−17.1	−7.37E-01	−4.62E-02	84.3	−3.87E-01	−10.8	−3.81	−1.68E-01	89.6	84.3
SE	20.7	2.52	2.77E-01	2.43E-02	21.8	6.09	4.29	8.54E-01	7.13E-02		
P-value	<0.01	<0.01	0.01	0.06	<0.01	0.95	0.01	<0.01	0.02		
Fat	1,624	−24.6	−4.04E-01	−1.24E-01	99.3	4.76	−15.7	−4.79	−1.64E-01	151	81.0
SE	29.9	3.86	4.43E-01	3.94E-02	30.7	8.53	5.99	1.26	1.14E-01		
P-value	<0.01	<0.01	0.36	<0.01	<0.01	0.58	0.01	<0.01	0.15		

[1]Standarized DIM = DIM ÷ 10 (weeks in the experiment) − 20 (average weeks in milk).

[2]Standarized CP = dietary CP% − 15.95 (average CP concentration).

[3]Linear effect.

[4]Quadratic effect.

[5]Cubic effect.

[6]RMSE = root mean square error with 496 observations corresponding to 62 cows during 8 consecutive weeks on the experimental diets.

[7]Milk NE_L (Mcal/d) calculated according to NRC (2001).

[8]Fat- and protein-corrected milk calculated according to IDF (2015).

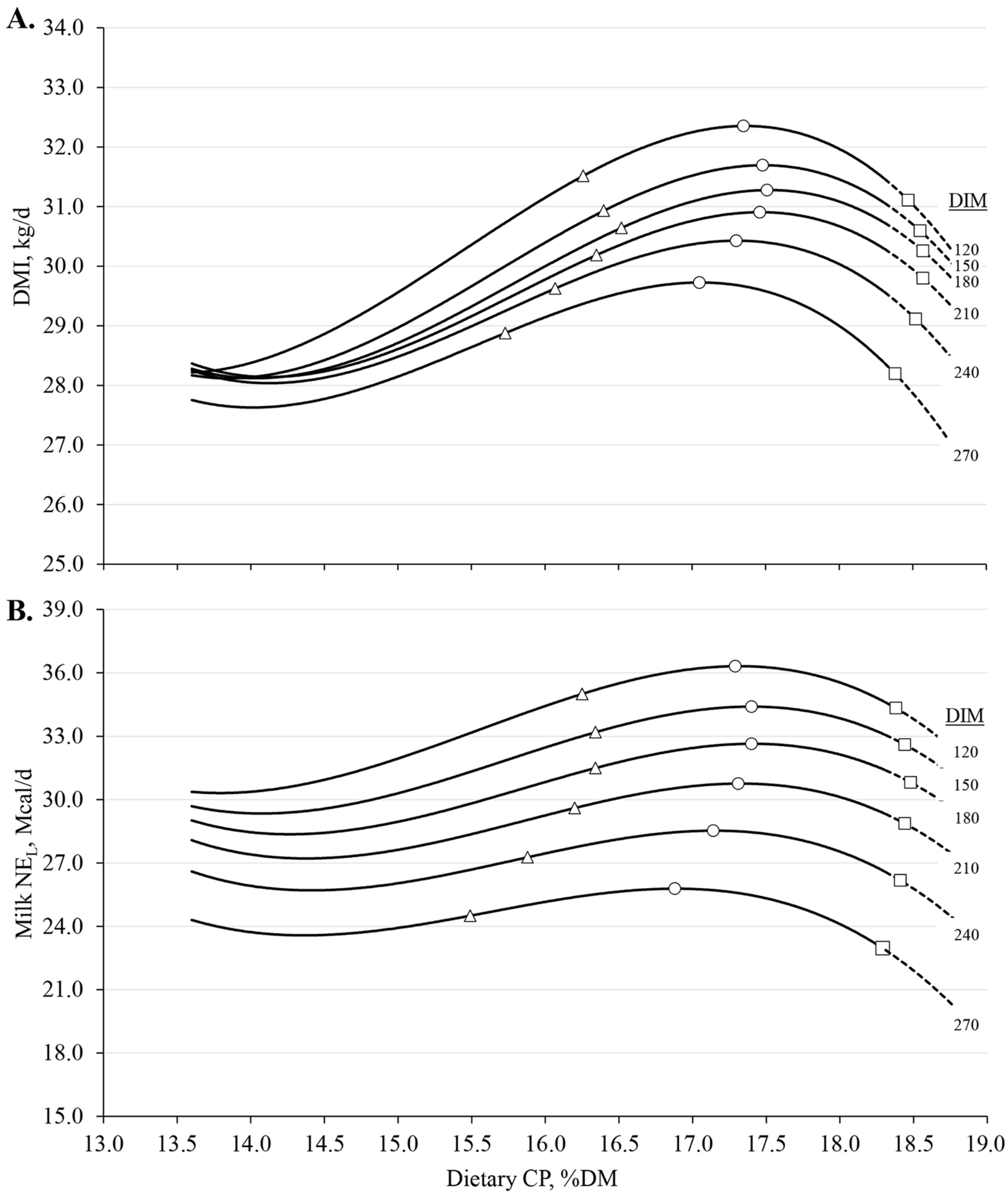

Figure 3. Predicted DMI (A) and predicted milk net energy for lactation (NE_L; B) in response to dietary CP% and DIM using parameter estimates derived from a multiple regression predictive model. Solid lines represent the range of dietary CP fed in this study. The dotted lines represent dietary CP outside the range fed in this study. The circles indicate the level of dietary CP associated with the predicted maximum response. The triangles and squares indicate the lower and upper limits of the 95% CI of the predicted maximum response, respectively. Predicted responses were based on 496 observations corresponding to 62 cows during 8 consecutive weeks on the experimental diets. Parameter estimates for these predicted responses are presented in Table 5.

CONCLUSIONS

Although many studies have evaluated the independent effects of CP or DIM, in this study, we observed significant interactions between dietary CP concentration and stage of lactation. Due to these interactions, caution is recommended when conducting, analyzing, and interpreting future studies on dairy cow protein nutrition across disparate DIM. Regression analysis indicated that CP ranging from 16.3 to 17.4% would maintain DMI and milk NE_L output in early and mid-early lactation. However, CP could be reduced to between 15.7 and 17.1% when cows progressed to late lactation. Our research suggests that there are distinct ranges in dietary CP concentration to maintain cow performance at each stage of lactation.

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