Dietary protein oscillation: Effects on feed intake, lactation performance, and milk nitrogen efficiency in lactating dairy cows

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ABSTRACT

Limited research with growing ruminants indicates that oscillating (OS) dietary crude protein (CP) concentration may improve nitrogen use efficiency (NUE). Our aim was to determine if a total mixed ration (TMR) based on OS CP (48-h phases of 13.4% and 16.5% CP, respectively) would increase NUE of lactating dairy cows compared with a static CP TMR (ST; 14.9% CP). The experiment was a randomized complete block design with 50 cows [150 ± 61 (mean $\pm$ SD) d in milk]. Cows were blocked by parity, days in milk, and milk protein yield. On average, diets were equal in composition over the total experiment. Cows were milked twice daily, and 8 milk samples were collected in each 4-d period. Each 48 h of low-CP (LP) and high-CP (HP) TMR offered to OS cows corresponded to milk collected at milkings 1 to 4 and 5 to 8, respectively. Dry matter intake (mean = 25.5 kg/d for both treatment groups); yields of milk (mean = 31.5 kg/d for both treatment groups), protein, fat, lactose, and fat- and protein-corrected milk (mean = 33.6 kg/d for both treatment groups); and milk concentration of protein, fat, and lactose did not differ between treatments. However, milk urea concentration was higher for OS compared with ST (12.2 vs. 11.3 mg/dL). Body weight, body condition score, NUE, and feed efficiency were unaffected by OS. Apparent total-tract digestibility of dry matter (695 vs. 677 g/kg), organic matter (714 vs. 697 g/kg), CP (624 vs. 594 g/kg), neutral detergent fiber (530 vs. 499 g/kg), and starch (976 vs. 973 g/kg) were higher for OS than for ST cows. Cows in OS responded transiently, and regression analysis of differences within block over time revealed changes in yield of milk (-531 g/d), milk protein (-25.6 g/d), and milk lactose (-16.7 g/d) in LP. Opposite effects were observed for yield of milk ($+612$ g/d), milk protein ($+28.8$ g/d), and milk lactose

($+28.0$ g/d) during HP. Changes in concentrations of milk protein ($-0.050\%/d$), lactose ($+0.030\%/d$), and urea (-3.0 mg/dL per day) during LP, and in milk lactose ($-0.024\%/d$) and urea ($+4.3$ mg/dL per day) during HP, were observed. Milk yield, lactose yield, and protein yield were lower for OS than ST cows at the last milking of LP and at the first milking of HP. Milk urea concentration did not show such a lag and was lower in the last 2 milkings of LP, and higher in the last 3 milkings of HP, in OS compared with ST cows. Overall, performance and NUE were unaffected by OS treatment, but apparent total-tract digestibility and milk urea concentration increased, and transient effects on milk yield and composition occurred in OS cows.

Key words: digestibility, rumen synchrony, milk response, protein supply

INTRODUCTION

Improved utilization of dietary N in dairy cattle decreases the amount of N excreted, particularly N excreted as urinary N, and thus reduces environmental output of ammonia, nitrate, and nitrous oxide (Dijkstra et al., 2013; Montes et al., 2013). Among strategies to improve N utilization, efforts to synchronize dietary energy and protein to improve production and efficiency have resulted in limited success (Cabrita et al., 2006; Reynolds and Kristensen, 2008). In contrast, several experiments have reported improved N retention in beef cattle and growing sheep when feeding protein supplements infrequently or when offering diets that oscillate in dietary CP concentration compared with supplying a constant dietary CP concentration. Cole (1999) fed static (12.5% CP) or oscillating (10.0 and 15.0% CP) dietary CP on a 24- or 48-h basis to growing lambs and reported a 38% increase in N retention for the 48-h oscillating-fed lambs compared with static-fed lambs when supplemental N was provided by cottonseed meal. However, there were no improvements with oscillating feed when a blend of cottonseed meal and dietary urea was used. Archibeque et al. (2007b) fed a static diet or an oscillating diet on 48-h phases to wethers and

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reported a higher N retention for oscillating diets (6.7 g/d) than for static diets (4.0 g/d), and a trend for an increased urea recycling to the portal-drained viscera. Wickersham et al. (2008) fed casein to steers, either daily in amounts of 61 or 183 mg of N/kg of BW or every third day in amounts of 183 (i.e., 3×61) and 549 (i.e., 3×183) mg of N/kg of BW per supplementation event. At the lowest supply of N, N retention tended to be greater for steers fed casein every 3 d than for steers fed casein daily (26.7 and 17.0% of intake, respectively). However, such a tendency was not observed at greater N supply despite the increase in urea gut entry. Doranalli et al. (2011) reported that lambs fed an oscillating diet compared with a static dietary CP showed higher N retention values (on average 32.1 vs. 23.6% of N intake, respectively) and higher ADG (340 vs. 276 g/d, respectively). Reported improvements in N retention were partially mediated by increased urea transport into the rumen during the low-CP phase, as measured by the Ussing chamber technique (Doranalli et al., 2011). Together, these studies indicate that urea recycling may at least partly explain improvements in productivity or efficiency when oscillating dietary CP. For urea recycling to be effectively upregulated during oscillation, frequency of oscillation should be similar to gastrointestinal tract mean retention time (MRT; Cole, 1999). This would allow for a concurrent relative excess of postruminal N absorption and deficiency in ruminal N during days of feeding the low-CP diet, resulting in a simultaneous increase and reduction in concentrations of plasma urea and ruminal ammonia, respectively, conditions known to stimulate urea flux into the rumen (Kennedy and Milligan, 1980; Abdoun et al., 2006). Tebbe and Weiss (2020) demonstrated that dairy cows receiving a diet that oscillated between 11.9 and 16.2% CP on a 24-h basis performed similarly in nitrogen use efficiency (NUE; milk N/N intake) and yields of milk, milk protein, and ECM to cows receiving a static diet (14.1% CP). A trend for an increase in N digestibility, greater MUN, and urinary N excretion (as % of N intake) in oscillating cows occurred, despite a lower N intake (Tebbe and Weiss, 2020). Based on improvements in production or efficiency with growing ruminants using 48-h periods of oscillations (Cole, 1999; Archibeque et al., 2007b; Doranalli et al., 2011), MRT measured in lactating dairy cows (marginally lower than 48 h, Warner et al., 2013), and lack of improvement in NUE when CP concentration was oscillated on a 24-h basis (Tebbe and Weiss, 2020), we hypothesized that a 48-h period of oscillation may be a suitable dietary strategy to improve NUE in lactating dairy cows. Our objective was to determine if an oscillating diet (OS; 48-h phases of 13.4 and 16.5% CP, DM basis,

respectively) compared with a static diet (ST; 14.9% CP) would increase NUE in lactating dairy cows.

MATERIALS AND METHODS

This study was conducted between March and April of 2019 at the Dairy Research Facility of Trouw Nutrition Research and Development (Kempenshof, Boxmeer, the Netherlands). The study complied with the Dutch Act on Animal Experiments in accordance with European Directive 2010/63/EU, and was approved by the Central Committee of Animal Experiments (the Hague, the Netherlands).

Animals and Experimental Design

Effects of ad libitum intake of a TMR based on dietary protein concentration oscillating in time [OS; 48-h phases each of 13.4% and 16.5% CP (DM basis), respectively] or static protein concentration (ST; 14.9% CP), were evaluated using 52 Holstein-Friesian dairy cows (150 ± 61 DIM at the start of the experiment; 18 primiparous and 34 multiparous of 3.0 ± 1.4 lactations) in a randomized complete block design. A schematic overview of the experiment is presented in Figure 1. The first 23 d served as covariate period, and experimental diets were then fed from d 24 to 51. Cows were fed daily during evening milking (at ~1630 h) and again at ~1100 h, and dietary changes to the OS treatment (i.e., at 48-h intervals) were applied at evening milking. Thus, a total of 4 morning milkings (M1, M3, M5, and M7) and 4 evening milkings (M2, M4, M6, and M8) were used to evaluate the effect of experimental diets within a 96-h period.

During the covariate period, all cows were fed a TMR diet with 14.9% CP (ST diet). Milk composition data collected during morning milkings of d 9, 11, 16, and 18, and evening milkings of d 8, 10, 15, and 17 were used to calculate milk covariates per cow. Similarly, DMI data from d 8 to 21, obtained with 28 roughage intake control (RIC) feed bins (Insentec), were used to compute a DMI covariate per cow. Cows were blocked according to parity, DIM, and milk protein yield, and assigned randomly to 1 of the 2 experimental groups within a block (26 blocks in total). Cows were then assigned to their respective RIC feed bins on the morning of d 22 to allow cows to learn which feeders they were able to access. All cows within a specific treatment had access to all RIC bins that were allocated to that respective treatment diet. The TMR was a homogeneous mixture obtained after mixing for approximately 20 min in a TMR-mixer wagon. However, the potential of TMR selection by cows and subsequent

Figure 1. Design of the experiment evaluating effects of dietary CP concentration of TMR diets fed to cows in static (ST) or oscillating (OS) treatments during the covariate (d 1–23; diets of both ST and OS treatments had targeted dietary CP concentration of the static diet) and experimental (d 24–51) periods. Fresh TMR was mixed and delivered during evening milking (~1630 h) and again at ~1100 h. Each day represents a 24-h period starting and ending at ~1630 h. Milkings 1, 3, 5, 7 and 2, 4, 6, 8 correspond to a.m. and p.m. milking times, respectively. Shaded areas represent days on which milk yield and milk composition data were collected.

variable nutrient composition of the TMR consumed between cows within the same treatment presents a potential limitation. Starting from evening milking of d 23, dietary treatment TMR were mixed and delivered to respective RIC bins. The OS treatment comprised 48 h of a low-CP TMR (**OS-L**; 13.4% CP on DM basis), followed by 48 h of a high-CP TMR (**OS-H**; 16.5% CP on DM basis), which was repeated every 4 d. To isolate the effect of oscillation from any dietary difference in the TMR composition between treatments, a common basal roughage mix (excluding concentrates) was mixed for both treatments, and 50% of the roughage mix was used for ST and OS treatments, respectively. The basal roughage mix was then mixed with 2 concentrates formulated with different CP concentrations as follows: a low-CP concentrate used for the OS-L diet, and a high-CP concentrate used for the OS-H diet. For the ST diet, an equal proportion of the low-CP and high-CP concentrates was used. From d 23 to 51, cows received their assigned TMR (either ST or OS) diets as described above in 7 periods of 4-d oscillations.

One cow was removed from the data set due to repeated mastitis and antibiotic treatment, and another was removed due to extreme increases and reductions in BW and milk yield. In both cows, respective prob-

lems were already observed during the covariate period and were thus assumed not to be related to treatments.

Housing

Cows were housed in a freestall barn with free access to fresh water and electronic access to their RIC boxes. Each RIC box was clearly marked for feeding purposes, and each treatment had 2 adjacent RIC boxes, followed by 2 adjacent RIC boxes of the alternative treatment to prevent potential confounding effects of location on feed intake. A total of 28 RIC boxes were used for the experiment. For every visit of a cow to a feeder, start and end weight of the feeder were recorded. Leftover feed was removed daily, and intake control boxes were checked daily for correct functioning.

Diet Formulation

Ingredients used to determine TMR composition were collected on d 43, 44, and 45 of the experiment because this was adjacent to the period of fecal collection used for digestibility calculations. Samples were frozen immediately at -18°C until analysis. The compositions of the ST, OS-L, and OS-H diets are presented in Table

1. Diets were formulated to be relatively deficient in RDP compared with rumen fermentable energy, as evidenced by the slightly negative average rumen protein balance (-10 g/kg of DM). This was intended to stimulate urea recycling, as urea flux into the rumen is positively related to fermentable energy and negatively related to rumen ammonia concentration (Kennedy and Milligan, 1980; Abdoun et al., 2006). From d 32 onward, concentrates contained 0.25% titanium dioxide as an inert marker to estimate the apparent total-tract digestibility.

Measurements

Cows were milked twice daily at ~ 0530 h and ~ 1630 h, and milk yield was recorded electronically. Milk samples were obtained via automatic samplers in the milking parlor that collected a fixed volume of milk per kilogram produced during morning and evening milkings of d 32 to 35, 40 to 43, and 48 to 51 of the experiment (Figure 1), representing the third, fifth, and seventh 4-d periods of oscillation, respectively. Samples were collected per cow and per milking into tubes containing sodium azide, stored at 4°C , and analyzed within 3 d. The BW and BCS were electronically recorded daily after p.m. milking when cows exited the milking parlor. The BCS recording was done with the DeLaval body condition scoring camera system. Fecal grab samples were collected from all cows on d 44 and 46 (0700 and 1300 h), and d 45 and 47 (0900 and 1500 h), frozen immediately at -18°C , and composited by cow to create a single composited fecal sample per cow per 4-d period. The RIC bins were filled with fresh TMR twice rather than once daily to stimulate a more gradual feed intake over the day. However, the limited sampling frequency (8 samplings per cow over a single cycle of oscillation) due to welfare and practical limitations may have affected digestibility estimates; therefore, those results should be interpreted accordingly.

Chemical Analysis

Chemical analyses were conducted at the Animal Nutrition Unit of Wageningen University and Research (Table 1). Samples of feed ingredients and feces were thawed at room temperature, dried at 70°C , and ground to pass a 1-mm screen using a Wiley mill (Peppink 100AN). The DM concentration of air-dried samples was gravimetrically determined by drying at 103°C until a constant weight was reached (ISO, 1999b). Ash was gravimetrically determined by combustion at a constant weight at 550°C (ISO, 2002). Determination of the total N concentration was done by combustion

Table 1. Ingredient and chemical composition (g/kg of DM, unless stated otherwise) of static (ST) or oscillating (OS; OS-L = low protein; OS-H = high protein) diets

Composition ¹	Diet		
	ST	OS-L	OS-H
Ingredient			
Corn silage	294	294	294
Grass silage	135	135	135
Grass hay	84	84	84
Corn, ground	152	170	134
Soy hulls	119	120	117
Wheat	103	121	85
Soybean meal	59	19	99
Soybean meal, protected ²	23	23	23
Limestone	9.4	9.5	9.3
Sodium bicarbonate	9.2	9.3	9.1
Monocalcium phosphate	4.2	5.1	3.4
Vitamin and mineral premix	3.4	3.5	3.4
Sodium chloride	2.0	2.0	2.0
Magnesium sulfate, anhydrous	2.6	3.0	2.1
Magnesium oxide	0.2	0.2	0.2
Live yeast ³	0.02	0.02	0.02
Nutrient composition			
DM, g/kg as fed	504	503	504
OM	925	926	924
CP	149	134	165
Crude fat	23	24	23
NDF	346	345	347
ADF	200	199	201
ADL	7	7	7
Starch	277	300	253
Total sugars	28	25	31
NE _L , MJ/kg of DM ⁴	6.89	6.88	6.89
DVE, g/kg of DM ⁵	91	85	98
OEB, g/kg of DM ⁶	-10	-21	2

¹Titanium dioxide was included at 0.25% of the concentrate fed from d 33 to 51.

²Formaldehyde-treated soybean meal (Mervobest, Agrifirm).

³Levucell SC 20, containing 2×10^{10} cfu/g (Lallemand).

⁴Calculated with the Dutch NE system (CVB, 2018) based on TMR ingredient composition.

⁵Intestinal digestible protein. Calculated with the Dutch DVE/OEB system (CVB, 2018) based on TMR ingredient composition.

⁶Rumen degradable protein balance. Calculated with the Dutch DVE/OEB system (CVB, 2018) based on TMR ingredient composition.

according to the Dumas principle (ISO, 2008), except in the case of corn silage, grass silage, and feces, where ammonia was first determined in fresh material and the ammonia-N concentration was added to the subsequent N concentration from the Dumas method in the dried samples. Spectrophotometric determination of ammonia in fresh samples was done as described by Nichols et al. (2018). Crude protein concentration was calculated as total N concentration $\times 6.25$. The NDF concentration of samples was measured according to Van Soest et al. (1991) after α -amylase treatment, without sodium sulfite but corrected for ash contamination. Acid detergent fiber and ADL analyses were performed according to Van Soest et al. (1991) and Robertson and Van

Soest (1981) using sulfuric acid, respectively. Starch was enzymatically determined according to ISO (2004). Determination of reducing sugars was based on Van Vuuren et al. (1993) but without using a segmented flow analyzer. Crude fat was determined gravimetrically after hydrolysis with hydrochloric acid and extraction with light petroleum at a boiling point of 40 to 60°C (ISO, 1999a). Titanium was determined after hydrolysis with concentrated sulfuric acid in the presence of a copper catalyst at 420°C and subsequent addition of peroxide. The resulting complex was spectroscopically determined at 408 nm (Nichols et al., 2018). Milk was analyzed for protein, fat, lactose, and urea concentration by mid-infrared spectroscopy (ISO 9622/IDF 141; IDF, 2013) using a MilkoScan 7 RM (Foss).

Calculations and Statistical Analysis

Milk yield and milk composition data were calculated for the actual days during which milk composition data were collected (i.e., d 32–35, 40–43, and 48–51 of the study) and corrected by cow for the milk yield and composition observed during the covariate period in the statistical analysis. Apparent total-tract digestibility of DM, OM, CP, NDF, crude fat, and starch was calculated by the marker ratio technique using titanium concentration measured in feed and feces samples, using the following formulas:

$$\begin{aligned} \text{DM digestibility (g/kg)} &= \\ 1,000 - [1,000 \times (\text{Ti}_{\text{diet}}/\text{Ti}_{\text{feces}})]; \\ \text{Nutrient digestibility (g/kg)} &= \\ 1,000 - [1,000 \times (\text{Ti}_{\text{diet}}/\text{Ti}_{\text{feces}}) \\ &\times (\text{Nutrient}_{\text{feces}}/\text{Nutrient}_{\text{diet}})], \end{aligned}$$

where Ti_{diet} and Ti_{feces} represent concentration of titanium in the diet and feces, respectively (g/kg of DM), and $\text{Nutrient}_{\text{feces}}$ and $\text{Nutrient}_{\text{diet}}$ represent concentration of nutrient in the feces and diet, respectively (g/kg of DM).

All statistical analyses were conducted with PROC MIXED in SAS (SAS 9.4M6, SAS Studio, SAS Institute Inc.) with cow as experimental unit. Cow, treatment, block, and period were used as class variables. The model included the fixed effects of treatment, period, the interaction of treatment and period, and the associated covariate value, and block was included as random effect. Period was included in the model as a repeated statement, with cow within block as subject. Period comprised the average output for the variable in ques-

tion of a 4-d period per treatment (48 h of OS-L and 48 h of OS-H, or 96 h of ST, respectively). For apparent total-tract digestibilities, the model only included fixed effect of treatment and random effect of block. For all statistical analyses, covariance matrices tested included unstructured, autoregressive(1), heterogeneous autoregressive(1), compound symmetry, heterogeneous compound symmetry, Toeplitz, and heterogeneous Toeplitz. The covariance matrix with the lowest Bayesian information criterion output was selected for reporting purposes.

Nitrogen use efficiency (%) was calculated as $(\text{CP in milk}/6.38)/(\text{CP intake}/6.25) \times 100$, where N in milk and N intake refer to total composite N in milk and total composite N in feed per 4-d period, respectively. Fat- and protein-corrected milk (kg/d) was calculated as $[0.337 + 0.116 \times \text{milk fat (\%)} + 0.06 \times \text{milk protein (\%)}] \times \text{milk yield (kg/d)}$ (CVB, 2018), and feed efficiency was calculated as kilogram of fat- and protein-corrected milk (FPCM)/kilogram of DMI.

All values reported in Table 2 are based on average values recorded in each 4-d period, corresponding to the third, fifth, and seventh 4-d period of oscillations. All data reported in Figures 2, 3 and 4 are based on the differences (Y) between the within-block difference of the cow receiving OS and the cow receiving ST during the experimental period and the corresponding within-block difference during the covariate period, calculated as follows:

$$\begin{aligned} Y &= [\text{Output variable}_{\text{OS}} - \text{Output variable}_{\text{ST}} \\ &\quad (\text{within block, experimental period})] \\ &\quad - [\text{Output variable}_{\text{OS}} - \text{Output variable}_{\text{ST}} \\ &\quad (\text{within block, covariate period})]. \end{aligned} \quad [1]$$

This allowed relative changes in the OS treatment to be reported clearly without confounding natural variation in response parameters. For DMI, daily values were used because data recording was done on a daily basis, with each 24-h period starting and ending at 1630 h. For milk yield and composition, data from each milking during the third, fifth, and seventh 4-d period was used and averaged by M1 to M8, respectively. Within a 4-d period, diets in the OS treatment were switched from OS-L to OS-H during M4, and from OS-H to OS-L during M8. Therefore, M1 to M4 represented milk produced when OS cows consumed the OS-L diet, and M5 to M8 represented milk produced when OS cows consumed the OS-H diet.

Differences as calculated with model [1] were used to calculate the rate of change (slope) in milk yield,

milk component yields, and milk composition during M1 to M4 for the low protein (**LP**) phase, and M5 to M8 for the high protein (**HP**) phase, respectively. Rate of change for DMI was calculated in the same manner, except that day was the unit of measurement. Slopes were analyzed in PROC MIXED of SAS with time as a continuous fixed effect and block as a categorical random effect. If a value had a studentized residual of less than -3 or more than 3 , it was classified as an outlier and removed before statistical analysis. For all statistical analyses, differences were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

RESULTS

Intake, Milk Production, BW and BCS, Feed and Nitrogen Use Efficiency, and Apparent Total-Tract Digestibility

No period $\times$ treatment or treatment effects were present ($P > 0.10$) for any of the feed intake or milk production variables, except that OS cows produced milk with a higher milk urea (**MU**) concentration than ST cows (Table 2). Similarly, BW and BCS were not affected by period $\times$ treatment interaction or by treatment ($P > 0.19$). No period $\times$ treatment or treatment effects were present ($P > 0.22$) for feed efficiency and milk N efficiency. Apparent total-tract digestibility of

DM, OM, CP, NDF and starch improved ($P < 0.05$) with OS treatment, but crude fat digestibility was not affected by treatment ($P = 0.13$; Table 3).

Transient Changes in DMI and Milk Yield and Composition

Dry matter intake of OS cows tended to be lower than that of ST cows on the first day of 4-d periods, corresponding to the first day of consuming the OS-L diet, but did not differ on other days (Figure 2). Cows in the OS treatment had a significantly ($P < 0.05$) lower MU concentration at M3 and M4, and higher MU concentration at M6, M7, and M8 than ST cows (Figure 3). There was a tendency ($P < 0.10$) for lower milk fat and milk lactose concentration at M1 and lower milk fat concentration at M7 for OS compared with ST cows. Compared with ST cows, OS cows produced less milk and milk protein at M4 after 2 d on the OS-L diet ($P < 0.05$; Figure 4). There was a tendency ($P < 0.10$) for OS cows to have a lower milk lactose yield at M4; reduced milk, milk protein, and milk lactose yields at M5; and an increased milk protein yield at M7 relative to ST cows.

Regression analysis of milk yield and milk composition (Table 4) indicated a significant time effect ($P < 0.05$) on yields of milk, milk protein, and milk lactose during the LP 2-d phase. During the HP 2-d phase,

Table 2. Performance of lactating dairy cows ($n = 25$ /treatment) receiving TMR based on either static (ST; daily CP 149 g/kg of DM) or oscillating (OS; CP 134 g/kg of DM for 48 h, followed by CP 165 g/kg of DM for 48 h) dietary CP concentration

Item	Treatment			P-value		
	ST	OS	SEM	Treatment	Period	Period $\times$ treatment
DMI, ¹ kg/d	25.5	25.5	0.31	0.95	0.62	0.62
CP intake, ¹ kg/d	3.80	3.81	0.042	0.77	0.58	0.66
Yield ¹						
Milk, kg/d	31.5	31.5	0.34	0.99	0.43	0.88
Fat, g/d	1,384	1,382	23.4	0.97	0.15	0.17
CP, g/d	1,156	1,157	13.2	0.95	0.15	0.77
Lactose, g/d	1,449	1,437	17.0	0.63	0.16	0.91
FPCM, kg/d ²	33.6	33.6	0.45	0.91	0.18	0.32
Milk composition ¹						
Fat, %	4.46	4.41	0.045	0.46	0.48	0.10
CP, %	3.71	3.71	0.017	0.98	<0.01	0.77
Lactose, %	4.57	4.56	0.013	0.71	<0.01	0.94
Urea, mg/dL	11.3	12.2	0.30	0.01	<0.01	0.25
BCS ¹	3.15	3.13	0.011	0.19	0.55	0.73
BW, ¹ kg	674	678	2.9	0.29	0.02	0.87
Feed efficiency, ¹ kg of FPCM/kg of DMI	1.34	1.33	0.020	0.61	0.02	0.22
Milk N efficiency, ³ %	31.0	30.8	0.44	0.71	0.02	0.22

¹Data used for calculations from 3 periods, i.e., d 32–35, 40–43, and 48–51.

²FPCM (fat- and protein-corrected milk) calculated using the formula: $[0.337 + 0.116 \times \text{milk fat (\%)} + 0.06 \times \text{milk protein (\%)}] \times \text{milk yield (kg/d)}$ (CVB, 2018).

³Milk nitrogen efficiency (%) = $[(\text{milk protein output} / 6.38) / (\text{dietary CP intake} / 6.25)] \times 100$. Calculations based on total protein yield and total dietary protein intake in a period (see text for details).

milk, milk protein, and milk lactose yields increased significantly ($P < 0.01$). Milk fat concentration tended to increase ($P = 0.08$), whereas milk protein and MU concentration decreased ($P < 0.02$) and milk lactose concentration increased ($P = 0.01$) during the LP phase. During the HP phase, milk fat concentration tended ($P = 0.10$) to decrease, whereas milk protein concentration ($P = 0.06$) tended to increase. Milk lactose concentration decreased ($P = 0.05$) and MU concentration increased ($P < 0.01$) significantly during the HP phase.

DISCUSSION

We hypothesized that dietary CP oscillations would improve NUE in dairy cows, based on positive published results in growing ruminants using similar dietary protein oscillation strategies. However, OS and ST cows in the current experiment performed similarly and NUE was not improved. Data were analyzed using 2 approaches and will be discussed accordingly (i.e., discussion of overall effects of dietary CP oscillations followed by discussion of transient effects of dietary CP oscillations within 4-d periods).

Figure 2. Dynamics of differences in DMI between static (ST) and oscillating (OS) treatments on d 1, 2 (low-CP diet, light gray) and d 3, 4 (high-CP diet, dark gray). Values are calculated as $[(DMI_{OS} - DMI_{ST})_{\text{within block, experimental period}}] - [DMI_{OS} - DMI_{ST}]_{\text{(within block, covariate period)]}$. * $0.05 < P < 0.10$; tendency for difference between ST and OS on day indicated. Error bars indicate SE.

Overall Effects of Dietary CP Oscillations

Intake, Performance, and NUE. Effects of dietary CP oscillation in ruminants remain ambiguous, and reported effects on NUE range from considerably negative (Ludden et al., 2002a) to substantially positive (Cole, 1999; Archibeque et al., 2007b; Doranalli et al., 2011). Recently, Tebbe and Weiss (2020) demonstrated that dairy cows receiving an oscillating CP diet (11.9% and 16.2% CP, 24-h basis) performed similarly in NUE, milk protein, and ECM yield to cows receiving a static CP diet (14.1% CP), in agreement with the present experiment. However, their reported lower N intake by cows with oscillating diet contrasts with our lack of treatment effect on N intake. Time frequency of the oscillation was different between these experiments, but amplitude and concentration of dietary protein, phase of lactation, and parity were comparable between studies. Reduced N intakes may therefore be related to a shorter frequency of oscillation used by Tebbe and Weiss (2020). In line with this, in our experiment, DMI of OS cows tended to be smaller than that of ST cows in the first 24 h, but not the subsequent 24 h, within a 48-h phase of feeding LP. Several studies with sheep (Cole, 1999; Archibeque et al., 2007b; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011) report improved N retention or ADG when feeding oscillating CP diets based on 48-h phases. This contrasts with our results on a 48-h phase and those of Tebbe and Weiss (2020) on a 24-h phase. In steers, N supplementation every 3 d increased apparently absorbed N and tended to improve N retention compared with isonitrogenous daily supplementation at a rate of 61 mg of N/kg of BW per day, but not at rates 3 times greater (183 mg of N/kg of BW per day; Wickersham et al., 2008). Cows in our study received 96 mg of N/kg of BW per day more during the OS-H phase than during ST, which is more comparable in supplementation rate to the low than to the high level of CP oscillation of Wickersham et al. (2008). Our lack of increased NUE contrasts with the results of Wickersham et al. (2008). Together with the sheep studies mentioned previously and the study of Tebbe and Weiss (2020), this suggests that dairy cows, in comparison with sheep and steers, respond differently to infrequent or oscillating dietary CP regimens.

Time frequency of the dietary CP oscillation should consider MRT of digesta in the gut to allow for increased urea recycling to the rumen (Cole, 1999). It is possible that MRT in our experiment was lower than the 48-h phases used and that our frequency of oscillation was too long to allow for sufficient increases in urea recycling to enable improved microbial CP synthesis and subsequent improvements in production or ef-

iciency. Average MRT in dairy cows in an experiment by Warner et al. (2013) was 47 h, based on different types of markers and treatments, similar to our 48-h oscillation phase. Higher DMI relative to BW in our study compared with that of Warner et al. (2013; 3.8 vs. 2.9%, respectively) suggested that MRT was likely shorter than 48 h in our study. However, our reported DMI and BW are comparable to those of Tebbe and Weiss (2020), in which a 24-h phase of oscillation was used without treatment effects on productivity or NUE. This suggests that factors other than the extent

of time of oscillation may partly or fully explain the lack of treatment effects on productivity and NUE in our study.

It was previously suggested that differences in rumen escape properties of the supplements may affect the effectiveness of oscillating N supply (Collins and Pritchard, 1992). In our experiment, dietary CP was oscillated using both RDP and RUP; consequently, potential benefits of dietary CP oscillations may have been limited compared with using mainly RUP. Similarly, Tebbe and Weiss (2020) reported a lack of treat-

Figure 3. Dynamics of differences in milk composition between static (ST) and oscillating (OS) treatments for milkings 1 to 4 (low-CP diet, shaded light gray) and milkings 5 to 8 (high-CP diet, shaded dark gray). Values are calculated as $[(\text{Output variable}_{OS} - \text{Output variable}_{ST} \text{ (within block, experimental period)}) - (\text{Output variable}_{OS} - \text{Output variable}_{ST} \text{ (within block, covariate period)})]$. * $0.05 < P < 0.10$; tendency for difference between ST and OS for milking indicated. ** $P < 0.05$; significant difference between ST and OS for milking indicated. Error bars indicate SE.

Table 3. Total-tract apparent digestibility (g/kg) of nutrients in lactating dairy cows ($n = 25/\text{treatment}$) receiving TMR based on either static (ST; daily CP 149 g/kg of DM) or oscillating (OS; CP 134 g/kg of DM for 48 h, followed by CP 165 g/kg of DM for 48 h) dietary CP concentration

Component	Treatment		SEM	P-value
	ST	OS		
DM	677	695	3.9	<0.01
OM	697	714	3.8	<0.01
CP	594	624	3.9	<0.01
Crude fat	577	597	9.4	0.13
NDF	499	530	8.7	0.01
Starch	973	976	1.1	0.04

ment effect on NUE and used both RDP and RUP to oscillate diets. Based on these findings and increased NUE with higher RUP diets (Collins and Pritchard, 1992; Cole, 1999), increasing dietary RUP, rather than dietary RDP, may hold potential for future research. A specific focus on mechanisms responsible for potential differences in responses between RUP- versus RDP-based approaches is required.

Digestibility and Postabsorptive NUE. Increased CP digestibility in OS cows is consistent with similar experiments that observed improvements in CP digestibility with sheep [Kiran and Mutsvangwa, 2009 (trend for reduced fecal N); Doranalli et al., 2011] and

Figure 4. Dynamics of differences in milk yield and milk component yields between static (ST) and oscillating (OS) treatments for milkings 1 to 4 (low-CP diet, shaded light gray) and milkings 5 to 8 (high-CP diet, shaded dark gray). Values are calculated as $[(\text{Output variable}_{OS} - \text{Output variable}_{ST} (\text{within block, experimental period})) - (\text{Output variable}_{OS} - \text{Output variable}_{ST} (\text{within block, covariate period}))]$. * $0.05 < P < 0.10$; tendency for difference between ST and OS for milking indicated. ** $P < 0.05$; significant difference between ST and OS for milking indicated.

Table 4. Regression analysis of intrablock differences (slopes) for dairy cows ($n = 25/\text{treatment}$) receiving TMR based on either static (ST, 14.9% CP daily) or oscillating (OS, 13.4% CP for 48 h, followed by 16.5% CP for 48 h) dietary CP concentration for milkings 1 to 4 (low protein) and milkings 4 to 8 (high protein) phases

Parameter	Phase				SEM
	Low protein		High protein		
	Mean	<i>P</i> -value	Mean	<i>P</i> -value	
Yield ¹					
Milk, g/d	−531	<0.01	612	<0.01	164
Fat, g/d	1.6	0.91	20.3	0.18	14.9
Protein, g/d	−25.6	<0.01	28.8	<0.01	5.9
Lactose, g/d	−16.7	0.04	28.0	<0.01	7.9
Milk composition ¹					
Fat, %/d	0.12	0.08	−0.12	0.10	0.07
Protein, %/d	−0.050	<0.01	0.027	0.06	0.013
Lactose, %/d	0.030	0.01	−0.024	0.05	0.012
Urea, mg/dL per day	−3.0	<0.01	4.3	<0.01	0.4

¹Data used for calculations from d 32 to 35, 40 to 43, and 48 to 51.

steers (Archibeque et al., 2007a). However, other studies report unaffected (Collins and Pritchard, 1992; Cole, 1999; Ludden et al., 2002b; Cole et al., 2003; Menezes et al., 2019) or decreased CP digestibility (Ludden et al., 2002a; restricted feeding) when feeding oscillating compared with static CP diets. Inconsistent effects of dietary CP oscillations on CP digestibility may be due to different ingredients or nutrients used when oscillating CP, and higher CP digestion may be observed when oscillating primarily RUP concentrations (Collins and Pritchard, 1992; Tebbe and Weiss, 2020).

Increased NDF digestibility in OS cows is consistent with similar findings in growing sheep (Kiran and Mutsvangwa, 2009). As urea has a rumen-buffering effect (Kertz, 2010), a potential increase in urea recycling to the rumen during the OS-L phase may have elevated rumen pH or reduced ruminal pH fluctuations and explain the observed increase in NDF digestibility in OS cows. Kiran and Mutsvangwa (2009) reported increases in excretion of allantoin and uric acid in oscillating lambs, and Doranalli et al. (2011) observed higher rumen pH and lower rumen ammonia levels in the low-CP relative to the high-CP phase of the oscillating CP treatment, with an overall increase in ruminal VFA concentrations in the oscillating compared with static treatment. Based on these findings, it is conceivable that improved ruminal conditions for microbial fermentation may at least partly explain the observed improvements in digestibility of NDF and other nutrients in OS cows of the current study. Improved digestibility without concurrent increases in productivity or BW indicated that postabsorptive losses were elevated in OS cows. Farmer et al. (2004) reported a reduced efficiency of digested N use for retention when steers consuming a tallgrass prairie hay basal diet were supplemented isonitrogenously with a high-CP concentrate twice per

week instead of daily, supporting our results of lower efficiency of digestible N for productive purposes in the OS treatment. Tebbe and Weiss (2020) reported reduced N intakes, increased CP digestibility, and similar digestible N intakes for oscillating compared with static cows, but urinary N as proportion of digestible N was significantly increased and productive N (milk N + retained N as proportion of digested N) was decreased for oscillating cows. As MU reflects blood urea (BU) concentration (Spek et al., 2013), we expect that in the present experiment, BU was increased in OS cows due to increased digestible N, and that extra absorbed N was excreted as urinary N, resulting in reduced postabsorptive efficiency in OS cows. However, it is not clear which mechanisms may have contributed to the reduced postabsorptive efficiency in response to OS dietary CP.

Transient Effects of Dietary CP Oscillations

Effects on Intake by Day of Oscillation. A trend for lower DMI during the first day of LP (Figure 2) is consistent with studies that report a positive association between CP concentration of the diet and DMI, and one of the mechanisms responsible may be the reduced hypophagic effects of propionate when dietary starch is replaced by protein (Allen, 2000). As protein in the OS-H diet was replaced by starch in the OS-L diet, an increase in propionate production in the rumen may have had temporary hypophagic effects on OS cows. A shortage of RDP may have occurred during the LP phase in OS, as suggested by a negative RDP balance (−21 g/kg of DM) with an associated negative effect on digestibility in the rumen and on DMI (Reynal and Broderick, 2005). However, this mechanism is not supported by the increased total-tract digestion of nutrients in the OS diet.

Effects on Milk Yield and Composition by Milking. Lower milk and milk protein yields for OS cows compared with ST cows at M4 and a trend for lower milk and milk protein yields at M5 (Figure 4) indicated that the OS-L diet affected milk yield with a delay of approximately 48 h from initial feeding, whereas a lag of about 36 h occurred before milk protein yield was increased (at M7; tendency) on the OS-H diet. A trend for reduced milk lactose yields at M4 and M5 for OS cows may be explained by a reduced metabolizable protein supply with the OS-L diet, resulting in reduced rates of hepatic AA deamination, gluconeogenesis, and lactose synthesis (Daniel et al., 2016; Loncke et al., 2020). This is supported by lower MU at M3 and M4 and higher MU at M6, M7, and M8 for OS cows compared with ST cows (Figure 3). Differences between OS and ST cows at peak MU levels were approximately 3 times larger than at nadir and agree with the increased average MU levels and postulated increased hepatic deamination in OS cows. A greater MU concentration in OS cows is consistent with larger highly labile protein reserves (Bannink et al., 2006; Spek et al., 2013), which ruminants may rely on during temporary dietary protein deficiencies (Reynolds and Kristensen, 2008). The trend for a decreased milk fat and milk lactose concentration during M1 and trend for decreased milk fat concentration during M7 may be explained by the diluting effects of a higher milk yield resulting from the OS-H diet.

Regression Analysis of Milk Yield and Composition by Phase

During the LP phase, milk, milk protein, and milk lactose yields decreased, and they increased again during the HP phase (Table 4). This transient behavior indicated that cows were able to respond within 48 h in synthesis and secretion into milk of these components, which is consistent with similar observations for yield of milk and milk protein in the study of Tebbe and Weiss (2020), where 24-h phases of oscillation were used. In contrast to our study, milk lactose yield did not differ between LP and HP in the study of Tebbe and Weiss (2020) and may be related to the shorter phases of oscillation used in that study. Notably, milk protein concentration in our study decreased and increased during LP and HP, respectively, which indicated that milk protein synthesis responded more rapidly than milk production as such. A lower milk and milk protein yield for OS cows during LP and increased milk and milk protein yields during HP is in agreement with the general positive association between increased dietary or metabolizable protein supply and yields of milk and milk protein at levels of dietary CP used in our experi-

ment (Daniel et al., 2016). The transient behavior of lactose concentration was opposite to that of protein concentration in milk. An increase in milk protein concentration due to increased MP supply has been associated with a decreased milk lactose concentration. As discussed by Nichols et al. (2019), milk casein and salt concentration are positively related, which may be explained by elevated AA flux into mammary gland cells through Na^+ dependent transporters. Given the role of lactose as an important osmotic regulator, this may explain this opposite transient behavior of milk protein (and mineral) concentration and milk lactose concentration. A tendency for an increased milk fat concentration during LP and a tendency for a decreased milk fat concentration during HP, without significant changes in fat yield, indicated a dilution effect of fat with increasing milk yield and vice versa. Consistent with this, milk fat yield was not different between LP and HP phases in the study of Tebbe and Weiss (2020). A high dietary energy-to-protein ratio, or the opposite (a low dietary energy-to-protein ratio), will decrease and increase MU levels, respectively. Milk urea levels reflect BU levels and relative dietary protein shortage or excess (Spek et al., 2013) and are consistent with our findings of decreased and increased MU levels during LP and HP, respectively.

CONCLUSIONS

Cows fed a diet that oscillated between low (13.4%) and high (16.5%) dietary CP (DM basis) every 48 h performed similarly to cows fed a static CP (14.9%) diet. Milk production and composition (except urea concentration), BW, BCS, and efficiency of N and feed use were not affected by treatment, but cows fed the OS diet had increased average MU concentration and nutrient digestibility compared with ST cows. Despite limited overall effects of dietary CP oscillations, transient effects of changes in dietary CP concentration on milk yield and composition were observed in cows fed the OS diet, indicating that dietary CP changes were large enough to obtain transient differences, but that compensatory mechanisms were able to mitigate potential negative consequences over 4-d periods. Based on transient effects observed in our experiment and on other research findings, we speculate that the use of primarily RUP sources to oscillate dietary CP may hold potential for future research to increase NUE in lactating dairy cows.

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