Effects of oscillating dietary crude protein concentrations on production, nutrient digestion, plasma metabolites, and body composition in lactating dairy cows

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ABSTRACT

We hypothesized that dairy cows fed oscillating metabolizable protein (MP) and crude protein (CP) concentrations on a 24-h frequency for a diet formulated to be below MP requirements would use N more efficiently (i.e., increased milk protein yields and less manure N) without increasing mobilization of body protein stores than would cows fed the same deficient MP diet continuously, although both treatments would on average have equal MP concentrations. In a randomized block design, 30 Holstein cows (119 ± 21 d in milk; 667 ± 69 kg of body weight) were blocked according to milk yield within a parity (3 primiparous and 7 multiparous blocks) and fed 1 of 3 treatments: (1) diet with 16.2% CP (109% of MP requirements) fed continuously (109MP), (2) diet with 14.1% CP (95% of MP requirements) fed continuously (95MP), or (3) diets oscillating on a 24-h cycle from the 109MP diet and a diet with 11.9% CP (~78% of MP requirements) such that average CP and MP concentration would be the same as 95MP (OSC). Dry matter intake was similar between 109MP and 95MP (22.9 vs. 23.2 kg/d) but tended to be lower for OSC (22.2 kg/d) compared with 95MP. Milk yield was greater for 109MP compared with 95MP (36.6 vs. 35.1 kg/d) and similar between 95MP and OSC (35.3 kg/d). Milk protein and energy-corrected milk yields were similar among treatments. Milk urea N (MUN) concentration was higher for 109MP compared with 95MP (12.8 vs. 10.2 mg/dL), and tended to be higher for OSC (10.9 mg/dL) compared with 95MP. Higher MUN concentration for OSC occurred despite lower N intake (474 vs. 512 g of N/d) and similar milk N outputs compared with 95MP (164 vs. 179 g/d). On days when cows on OSC were fed high versus low MP diets, yields of milk (34.8 vs. 36.3 kg/d) and milk protein (1.0 vs. 1.1 kg/d) and MUN concentration (9.3 vs. 12.5 mg/dL) followed the oscillation pattern but lagged the change in diet

CP by 1 d, whereas dry matter intake, yields of milk fat, plasma energy metabolites, AA, and 3-methyl-His were similar between days. Nutrient digestibility was similar for major nutrients across treatments except for CP, which was greater for 109MP (65.2%) and OSC (65.3%) compared with 95MP (61.7%). Compared with 95MP, OSC did not increase milk N relative to N intake (averaged 0.35 g of milk N/g of N intake) or N balance; however, urinary N output was increased for OSC versus 95MP (0.32 vs. 0.24 g of urine N/g of N intake). Body composition estimated using urea dilution was similar across treatments, and all cows accreted lipid and energy during the trial. Empty body CP did not change over the 50-d treatment period. Overall, greater CP digestion, urinary N excretion, and MUN concentrations with lesser N intake and similar milk N outputs for OSC compared with 95MP suggests that the lower energy intake by OSC cows may have limited potential responses to altered N metabolism.

Key words: nitrogen metabolism, amino acids, body composition, manure nitrogen

INTRODUCTION

Moderately decreasing dietary CP (e.g., 16 to 14% CP) consistently improves nitrogen utilization efficiency (NUE; milk N/N intake) by dairy cows and can reduce feed cost but is rarely done on farms because lower CP concentrations often decrease milk and milk protein yields (Jonker et al., 2002; Huhtanen and Hristov, 2009). Reduced milk production when dietary CP is decreased can outweigh environmental gains from reduced manure N excretion at the individual farm level because globally more cows would be needed to produce the same quantity of milk (VandeHaar and St-Pierre, 2006).

Oscillating dietary CP concentrations from deficient to adequate levels may improve NUE and allow diets with lower average CP concentrations to be fed without reducing milk production. In growing wethers and steers, oscillating dietary CP concentrations (i.e., 10 to 15% CP) every 48 h improved NUE and maintained BW

Received March 29, 2020.

Accepted June 21, 2020.

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gain compared with animals continuously fed a higher average CP concentration (Cole, 1999; Archibeque et al., 2007a). Improved NUE when oscillating CP may be from less urinary N excretion and more N recycled to the rumen for microbial protein synthesis, which increases MP supply compared with continuously feeding a similar average CP concentration (Archibeque et al., 2007b; Doranalli et al., 2011).

Previously, our laboratory found oscillating CP every 48 h from 10.3 and 16.4% in dairy cows numerically decreased milk and milk protein yields compared with continuously feeding a 13.4% CP diet (Brown, 2014). However, results from Brown (2014) and a preliminary experiment we conducted suggested feeding a deficient CP concentration for 48 h may be too long, and the average MP concentration of the oscillation treatment was too deficient for maintaining milk production. Because manure N excretion and body composition were not measured in those studies, we do not know whether oscillating CP in dairy cows can improve NUE and whether improved NUE is caused by reducing manure N excretion or increased mobilization of body protein.

Our objective was to determine whether oscillating dietary CP and MP every 24 h would alter milk production, nutrient digestion, plasma metabolites, and body composition in lactating dairy cows. We hypothesized that oscillating the MP concentrations on a 24-h frequency for a diet that was formulated to be below MP requirements would improve NUE, increase milk and milk protein yields, and reduce manure N excretion without increasing mobilization of body protein stores compared with feeding a diet with a similar average MP concentration continuously.

MATERIALS AND METHODS

Cows and Treatments

All procedures involving animals were approved by The Ohio State University Institutional Animal Care and Use Committee (protocol #2018A00000040). Nine primiparous and 21 multiparous cows (119 ± 21 DIM at the start of the experiment) were used in a randomized block experiment. Cows were blocked according to parity and 10-d average milk yield (primiparous blocks: 34 ± 2.3 kg/d, 637 ± 43 kg of BW; multiparous blocks: 41 ± 4.0 kg/d, 683 ± 74 kg of BW). Limited available stalls required the trial to be conducted in 2 groups of 15 cows. Treatments were represented equally in each group. The experiment consisted of a 10-d covariate period and 50 d of treatment feeding for each group (group 1: May to July 2018; group 2: August to October 2018). Treatments (Tables 1 and 2) were (1) a diet that on average provided 109% of MP requirements (NRC,

Table 1. Ingredient composition of diets¹

Ingredient, % of DM	Treatment diet ²		
	High phase OSC	95MP	Low phase OSC
Corn silage	40.5	40.5	40.5
Alfalfa silage	14.0	14.0	14.0
Corn grain	19.5	21.7	23.9
Soybean meal, 48% CP	16.1	11.1	6.1
Soy hulls	6.6	9.1	11.7
Animal/vegetable fat	0.51	0.51	0.51
Calcium soaps of FA ³	0.86	0.86	0.86
Limestone	0.23	0.12	—
Dicalcium phosphate	0.41	0.55	0.68
Dynamate ⁴	0.11	0.15	0.19
Potassium carbonate ⁵	—	0.12	0.23
Minerals/vitamins ⁶	1.4	1.4	1.4

¹The average ingredient composition fed to the 2 groups of cows used in the experiment (n = 15 cows per group).

²Treatments were (1) providing 109% of MP requirements continuously (109MP), (2) providing 95% of MP requirements fed continuously (95MP), or (3) 24-h oscillations from 109MP and a diet providing 78% of MP requirements (OSC). The 109MP treatment was fed the high phase OSC diet continuously. The 95MP treatment is the average of the high and low phase of OSC diets and fed continuously. The OSC treatment oscillated from the high phase OSC diet to low phase OSC diet on a 24-h basis.

³Megalac, Church & Dwight Co. Inc., Piscataway, NJ. FA = fatty acids.

⁴Mosaic Co., Plymouth, MN.

⁵DCAD Plus, Church & Dwight Co. Inc..

⁶Premix contained 49.0% trace mineral salt (Morton Salt Inc., Chicago, IL), 7.1% magnesium oxide (Animag Prilled 30/100, Martin Marietta Magnesia Specialties LLC, Baltimore, MD), 11.9% sodium selenate premix (200 mg Se/kg), 0.25% copper sulfate, 0.28% Zinpro 120 (120 g Zn/kg, Zinpro Corp., Eden Prairie, MN), 0.7% vitamin A (30 kIU/g), 2.2% vitamin D (3 kIU/g), and 3.3% vitamin E (44 kIU/kg), 24.7% biotin premix (220 mg/kg), and 0.48% Rumensin-90 (Elanco Animal Health, Greenfield, IN).

2001) fed continuously (**109MP**), (2) a diet that on average provided 95% of MP requirement fed continuously (**95MP**), or (3) 24-h oscillations from the 109MP diet and a diet that provided 78% of MP requirements (**OSC**). The CP and MP concentrations of OSC were on average equal to 95MP. For the covariate period, all cows were continuously fed the 109MP diet. Cows were housed in individual tiestalls and were fed once daily (0700 h) as a TMR with a refusal rate of 3 to 5%.

For each group, 2 diets were formulated to administer treatments: a high and a low phase OSC diet (Tables 1 and 2). The high phase OSC diet was also the 109MP treatment diet and formulated to meet 105% of the NRC (2001) MP requirement (actual was 109%) for cows at the start of the experiment. The low phase of OSC diet provided about 80% of the MP requirement (actual was 78%) and reduced milk yields 3.2 ± 0.98 kg/d based on a preliminary experiment. The 95MP treatment diet was a 50:50 mix of the high and low phase OSC diets and met 95% of MP requirements.

Table 2. Nutrient composition of diets¹

Nutrient ³	Diet ²		
	High phase OSC	95MP	Low phase OSC
DM, %	60.0	59.2	58.8
OM, % of DM	93.5	93.5	93.6
CP, ⁴ % of DM	16.2	14.1	11.9
RDP, ⁵ % of DM	10.4	9.1	7.8
MP, ⁵ % of DM	11.6	10.1	8.7
RUP, ⁵ % of DM	5.4	4.8	4.2
Forage NDF, % of DM	22.4	22.4	22.4
NDF, % of DM	31.3	33.1	35.0
NDF _{om+cp} , ⁶ % of DM	29.7	31.4	33.1
NDICP, % of DM	1.06	1.16	1.26
Starch, % of DM	26.0	27.6	29.1
LCFA, % of DM	3.51	3.48	3.45
ROM, % of DM	16.5	15.3	14.2
ROM _{om+cp} , ⁶ % of DM	18.1	17.1	16.0
NE _L , ⁵ Mcal/kg	1.63	1.61	1.59
Ca, % of DM	0.88	0.86	0.85
P, % of DM	0.39	0.40	0.41
Mg, % of DM	0.26	0.25	0.24
K, % of DM	1.34	1.33	1.32
Na, % of DM	0.32	0.31	0.30
S, % of DM	0.22	0.22	0.21
Cu, mg/kg	16	15	14
Fe, mg/kg	249	271	292
Mn, mg/kg	66	64	62
Zn, mg/kg	78	76	74

¹The average ingredient composition fed to the 2 groups of cows used in the experiment (n = 15 cows per group).

²Treatments were (1) providing 109% of MP requirements continuously (109MP), (2) providing 95% of MP requirements fed continuously (95MP), or (3) 24-h oscillations from 109MP and a diet providing 78% of MP requirements (OSC). The 109MP treatment was fed the high phase OSC diet continuously. The 95MP treatment is the average of the high and low phase of OSC diets and fed continuously. The OSC treatment oscillated from the high phase OSC diet and low phase OSC diet on a 24-h basis.

³LCFA = long-chain fatty acids; residual organic matter (ROM) = 100 - %CP - %LCFA - %ash - %starch - %NDF.

⁴The SD of diet CP concentrations across week of treatment feeding was 0.71, 0.57, and 0.44 for the high phase of OSC, 95MP, and low phase of OSC diets, respectively. The SD was determined using the SD of ingredients and 100 permutations of a Monte Carlo simulation to estimate variance.

⁵Estimated using the NRC (2001) model with average DMI.

⁶NDF_{om+cp} = NDF - NDI-ash (ashed NDF residues) - NDICP (NDF residues analyzed for CP); ROM_{om+cp} = 100 - %CP - %LCFA - %ash - %starch - %NDF_{om+cp}.

The low phase OSC diet was made by replacing soybean meal in the high phase OSC diet with corn grain and soyhulls and balancing for minerals. We replaced both soyhulls and corn grain to reduce potential negative effects of high starch concentrations on milk fat synthesis. The composition of the alfalfa silage differed between groups (Table 3); therefore, ingredient composition of diets had to be altered slightly so that nutrient composition was the same across groups (Supplemental Tables S1 and S2; <https://doi.org/10.3168/jds.2020-18613>).

Measurements, Sampling, and Laboratory Analyses

For each cow, feed ingredient delivery and refusal amounts were weighed and recorded daily. Silages were sampled weekly, analyzed for DM (100°C for 48 h), and used to adjust diets for any changes in silage DM. Concentrates and silages were sampled twice per week and composited monthly (2 composite samples per group) for nutrient analyses. Refusal samples were taken once during the covariate period and every 2 wk during treatment feeding, analyzed for DM (100°C for 48 h), and used to calculate DMI. Cows were milked twice daily at 0400 and 1600 h, and milk yields were measured electronically (Afimilk, Kibbutz Afikim, Israel). Milk samples were collected weekly from 4 consecutive milkings for analysis of milk fat, true protein, and lactose (B2000 Infrared Analyzer, Bentley Instruments, Chaska, MN), and MUN concentration (Skalar SAN Plus segmented flow analyzer, Skalar Inc., Norcross, GA) by DHI Cooperative Inc. (Columbus, OH). The weighted average of a.m. and p.m. samples based on milk yield at sampling was used to calculate milk component yield for each day. Milk component yields measured on consecutive days were taken to capture each phase of oscillation (i.e., high and low).

On d 10 of the covariate period and on consecutive mornings during the middle (group 1: d 24 and 25; group 2: d 27 and 28) and end of treatment feeding (d 49 and 50), blood was collected approximately 1

Table 3. Nutrient composition of forages included in the diets¹

Nutrient ²	Corn silage	Alfalfa silage	
		Group 1	Group 2
DM, %	31.9	38.5	37.6
OM, % of DM	96.1	89.3	91.2
CP, ³ % of DM	7.30	22.8	17.6
NDF, % of DM	41.7	33.0	51.0
NDF _{om+cp} , ⁴ % of DM	40.4	30.7	48.4
NDICP, % of DM	0.74	1.56	1.80
Starch, % of DM	29.3	3.00	2.01
LCFA, % of DM	2.66	3.00	2.01
ROM, % of DM	15.1	29.0	18.3
ROM _{om+cp} , ⁴ % of DM	16.4	31.1	20.85
Ca, % of DM	0.26	1.47	1.27
P, % of DM	0.24	0.33	0.38
Mg, % of DM	0.13	0.29	0.29
K, % of DM	1.05	2.68	2.58

¹Group 1 and 2 received the same crop of corn silage (n = 8 samples) but each group received different cuttings of alfalfa silage (n = 4 samples per group).

²LCFA = long-chain fatty acids; residual organic matter (ROM) = 100 - %CP - %LCFA - %ash - %starch - %NDF.

³The SD of the CP in corn silage was 0.38, and for group 1 and 2 alfalfa silage was 1.03 and 1.34, respectively.

⁴NDF_{om+cp} = NDF - NDI-ash (ashed NDF residues) - NDICP (NDF residues analyzed for CP); ROM_{om+cp} = 100 - %CP - %LCFA - %ash - %starch - %NDF_{om+cp}.

h before feeding from the tail vein into sodium heparin vacutainers (BD Vacutainer, Franklin Lakes, NJ). Plasma was separated by centrifugation ($2,500 \times g$ at 4°C for 20 min) and frozen at -20°C before analysis. Analyses included albumin (Albumin LiquiColor No. 0285), total protein (Total Protein LiquiColor No. 0250), glucose (Glucose LiquiColor No. 1070, Stanbio Laboratory, Boerne, TX), fatty acids [NEFA-HR(2), Wako Chemicals, Richmond, VA], and insulin (#PI-12K Porcine Insulin RIA, EMD Millipore, St. Charles, MO). Subsamples of plasma collected from consecutive days at the end of treatment feeding were also analyzed for AA using liquid chromatography tandem MS (Heartland Assays LLC, Ames, IA).

For apparent nutrient digestibility and N balance, total output of feces and urine was measured on 24 cows (2 primiparous and 6 multiparous blocks) following methods described by Weiss et al. (2009). After at least 20 d on experimental diets, 2 blocks of cows were moved from tiestalls to digestion stalls. Because only 6 digestion stalls were available, 2 blocks of cows were moved for each of the 4 digestion trials (2 trials per group). Urine (pH kept below 3 by acidifying with 50% sulfuric acid), feces, milk, refusals, and feeds were sampled daily (refrigerated between days) and composited by wet weight across the 4-d collection period. In 3 of the 4 digestion trials (one set of samples were missed because of a scheduling error), daily subsamples of all feeds were taken and analyzed for CP (Kjeldahl N $\times$ 6.25; AOAC International, 2000; 984.13.4.09) to evaluate day-to-day variation in diet composition. To reduce diet sorting during total collection, feed delivery was reduced so that refusal rate was 1 to 3%. Daily water intake was measured with water meters connected to each individual water bowl.

Milk, acidified urine, and wet feces composite samples were analyzed for N (Kjeldahl method) immediately following each digestion period. Acidified urine was also frozen and later analyzed for creatinine (creatinine urinary assay no. 500701, Cayman Chemical Co., Ann Arbor, MI), uric acid (Uric Acid LiquiColor No. 1045, Stanbio Laboratory, Boerne, TX), allantoin (Chen, 1989), and carbon using an elemental analyzer (Flash 2000, Thermo Fisher Scientific, Waltham, MA) after oven-drying the urine (Morris et al., 2019a). Subsamples of concentrates and silages (digestion and monthly composite samples), refusal, and fecal composites were analyzed for DM (100°C for 48 h). The remaining silage, fecal, and refusal composite samples were frozen, lyophilized, and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA). Concentrates and ground samples of silages, feces, and refusals were assayed for DM (100°C for 24 h), ash (muffle oven at 600°C overnight), CP (Kjeldahl

N $\times$ 6.25), long-chain fatty acids (LCFA; Weiss and Wyatt, 2003), starch (Weiss and Wyatt, 2000) with modifications as stated in Tebbe et al. (2018), and NDF (Ankom200 Fiber Analyzer, Ankom Technology Corp., Fairport, NY) with sodium sulfite and amylase (Sigma A3306, Sigma Diagnostics, St. Louis, MO). The NDF residues were ashed (NDI-Ash; muffle oven at 600°C overnight) or analyzed for CP (NDICP; Kjeldahl N $\times$ 6.25). The NDI-ash and NDICP values were used to calculate NDF and residual organic matter (ROM, % of DM = $100 - \%CP - \%LCFA - \%ash - \%starch - \%NDF$) as ash-free, CP-free, or both to correct for double subtraction of ash and CP in ROM (Tebbe et al., 2017). Dried, ground feed samples were analyzed for minerals (OARDC STAR Laboratory, Wooster, OH) by inductively coupled plasma emission spectroscopy (Isaac and Johnson, 1985) after microwave digestion in nitric acid (Jones et al., 1991).

Body Composition

Body composition was estimated during the covariate period and 1 to 2 d after treatment feeding ended using the urea dilution method (Agnew et al., 2005). Cows were weighed the day before and day of infusion, and body condition (1 to 5 scale) was scored by 4 trained individuals. Approximately 4 h after feeding, cows were moved to a chute and restrained with a halter to expose the jugular vein. A $10\text{-} \times 10\text{-cm}$ area around the jugular vein was shaved, scrubbed twice with 7.5% povidone iodine and 70% alcohol, and an injection with 1.5 mL of 2% lidocaine-HCl was made subcutaneous and superficial to the jugular vein. The area was scrubbed again followed by a 1-cm incision made with a scalpel blade on the injection site and insertion of a catheter (14-gauge $\times$ 133-mm, 219 mL/min capacity; BD Angiocath, BD Infusion Therapy Systems, Sandy, UT) into the jugular vein. A small amount of blood was allowed to flush the tubing attached to the catheter before a baseline blood sample was taken. Using 200-mL syringes, cows were infused with a weighed amount of sterile, urea solution (0.9% NaCl and 20% wt/vol urea; reagent grade from Fisher Chemical, Fair Lawn, NJ). Infusion amount was 130 mg of urea/kg of BW (0.65 mL of solution/kg of BW) and was infused over 2 to 3 min. After infusion, the tubing was immediately flushed with heparinized saline (10 IU of heparin/mL and 0.9% NaCl), the catheter was removed, and the incision site was closed with tissue adhesive (Vetbond, 3M Animal Care Products, St. Paul, MN) and sprayed with aerosol bandage (AluShield, Neogen Corp., Lexington, KY). Cows were returned to tiestalls, and another blood sample was collected from the coccygeal vein promptly at 12 min

after infusion. All blood samples were collected into sodium heparin vacutainers and immediately placed on ice. Plasma was separated by centrifugation (described above) and frozen at -20°C until urea concentration was measured using a colorimetric method (SKU# UR1068, Randox Laboratories Ltd., Crumlin, UK).

Urea space volume was calculated as the quantity of urea infused divided by the difference in urea concentration from baseline and postinfusion plasma samples. Urea space volume, average body condition and BW, and the previous week's average milk yield were then used to calculate empty BW, CP, lipid, ash, gross energy, and water using equations 7d, 8, 9d, 11d, and 12, respectively, from Agnew et al. (2005). In addition to BW collected for body composition measurements, BW was measured on consecutive mornings on d 24 and 25 (group 1) and d 27 and 28 (group 2), averaged, and used for statistical analysis.

Calculations and Statistical Analyses

For statistical analyses, production measurements were averaged within cow for the covariate period and weekly for the treatment period (7 wk total). To evaluate the effect of oscillation phase on production and blood measurements, all measurements taken on days designated as high within the oscillation cycle were averaged within cow as were measurements on days designated as low. To assess insulin resistance of peripheral tissues, the homeostasis model assessment of insulin resistance (**HOMA-IR**) and the revised quantitative insulin sensitivity check index (**RQUICKI**) were calculated according to De Koster and Opsomer (2013). Insulin resistance was investigated because high protein diets have been associated with greater insulin resistance in humans (Rietman et al., 2014). Apparent digestibility and N metabolism measurements were calculated for each cow, and digestible energy (**DE**; Mcal/d) was estimated using intakes of digestible nutrients and enthalpies stated in Tebbe et al. (2018). Diet NE_L concentrations were estimated 2 ways. First, NE_L was calculated by subtracting estimated urine (Hoffmann and Klein, 1980) and methane energy (Nielsen et al., 2013) from DE (all expressed as Mcal/d), then multiplying by an assumed efficiency of 0.66 (Agnew and Yan, 2000) and dividing by daily DMI. Treatment NE_L was also calculated by summing estimated energy expenditures (i.e., maintenance, lactation, and BW changes) and dividing by DMI measured over the 50-d experiment: $\text{NE}_L, \text{Mcal/d} = 0.1 \times \text{BW}^{0.75} + \text{BW change} \times 5.6 + \text{milk energy}$, where maintenance energy is from Ellis et al. (2006) and BW and milk energies are from NRC (2001).

Two multiparous cows (one on 109MP and one on OSC) developed chronic mastitis during treatment feeding and were removed during the experiment; data were not used from either cow. All statistical analyses were made using PROC MIXED (version 9.4, SAS Institute Inc., Cary, NC). The model for average daily production, BW, and blood data included the fixed effects of treatment, week (repeated), their interaction, and the random effects of group, block within a group, block $\times$ treatment, and residual error. The model used to analyze the effect of oscillation phase included the fixed effects of treatment, OSC phase (i.e., high or low), and their interaction, the random effects of group, block within a group, cow within a group by treatment, and residual error. When the treatment by phase interaction was significant ($P < 0.10$), the SLICE option was used to determine whether phase had an effect within dietary treatment. For digestibility, balance, and body composition measurements, the model included the fixed effect of treatment and random effects of block and residual error. Body composition, blood metabolites (except AA concentrations), and production least squares means were covariate adjusted from measurements taken during the covariate period. The autoregressive covariance structure was used for repeated measurements based on lowest Bayesian information criterion. Denominator degrees of freedom were adjusted using the Kenward-Roger option. Two nonorthogonal contrasts were chosen a priori and used to evaluate effect of CP concentration (109MP vs. 95MP) and effect of oscillation (95MP vs. OSC). The homogeneity of treatment variances on production measurements was assessed using the GROUP option of the MIXED procedure. The Bayesian information criterion of production measurements and the interpretation of treatments were similar whether the variances were separated by treatment or combined. The highest standard error of the mean was reported for all measurements because of missing data.

RESULTS AND DISCUSSION

The low phase OSC diet had about 25% less CP and 15% less ROM, whereas NDF and starch concentrations were both about 12% greater compared with the high phase OSC (Table 2). Based on actual production and DMI, the 109MP, 95MP, and OSC treatments (on average) provided, respectively, 109, 95, and 95% of MP, and 99, 86, and 86% of RDP requirements (NRC, 2001). When cows on OSC were fed the low phase OSC diet and using the average production and DMI from that day, the diet provided 78% of MP and 73% of RDP requirements. As designed, the day-to-day coefficient

of variation in dietary CP was considerably higher for OSC (17.0%) compared with 95MP (3.4%) and 109MP (1.3%). The coefficients of variation were calculated from daily samples taken during the 4-d total collection experiments.

Production

Compared with 109MP, feeding 95MP continuously did not influence DMI, but as designed, reduced ($P = 0.02$) milk yield 1.5 kg/d (Table 4). As expected, production was similar for each phase (i.e., days when OSC were fed high or low phase OSC diets) for the 109MP and 95MP treatments ($P \geq 0.15$; Table 5). For OSC, DMI was numerically reduced ($P = 0.11$) and milk yield was similar to feeding 95MP. During the 4-d digestion trials, DMI was lower (Table 6) for OSC than 95MP. Within the OSC treatment, phase did not affect DMI; however, milk yield was 1.5 kg/d higher ($P < 0.01$) during the low phase compared with the high phase OSC diet.

In longer term experiments with growing ruminants, DMI has been similar (Ludden et al., 2002a; Doranalli et al., 2011) or tended to decrease (Doranalli et al., 2011) when oscillating CP concentrations every 48 h compared with feeding a similar average CP concentration continuously. Dietary factors that can affect DMI include RDP deficiencies (Oldham, 1984) and increasing inclusion rate of soyhulls (Ipharraguerre et al., 2002), which occurred when cows changed from high phase to low phase. Those dietary effects on DMI could

be mediated via reduced NDF digestibility; however, treatment did not affect NDF digestibility (Table 6). Supply of AA can also affect DMI (Lee et al., 2012) but treatments and day of oscillation phase had only minor effects on plasma AA concentrations (Table 7; Supplemental Table S3; <https://doi.org/10.3168/jds.2020-18613>). The reasons for why OSC likely reduced DMI compared with 95MP are not known.

Yields of ECM, milk fat, and milk energy as well as their concentrations in milk were not affected by treatment (Table 4). Milk protein concentration was reduced ($P < 0.04$) for both 109MP and OSC treatments compared with 95MP; however, milk protein yield was unaffected by CP concentration or oscillation ($P > 0.17$). Yield of lactose increased for 109MP but was similar between 95MP and OSC. Similar to milk yield, cows fed OSC had higher ($P = 0.04$) yield of milk protein and tended to have greater ($P = 0.10$) milk lactose yield during the low phase compared with high phase of the oscillation (Table 5). However, yields of ECM and milk fat were similar ($P \geq 0.51$) between the high and low days for OSC. Daily changes in milk protein yields but not for milk fat or ECM yields suggests MP supply was the limiting nutrient for milk production and that cows require a continuous dietary supply of MP. This means protein stores (e.g., skeletal muscle) cannot rapidly supply enough AA to spare a short-term deficiency in MP supply.

Milk urea N concentration was the only production response that had a treatment by week interaction ($P < 0.01$). At all weeks except wk 2 and 7, MUN con-

Table 4. Effects of static or oscillating CP concentrations on intake and production for the 50-d experiment

Item	Treatment ¹			SEM	P-value ²	
	109MP	95MP	OSC		109MP vs. 95MP	95MP vs. OSC
DMI, kg/d	22.9	23.2	22.2	1.08	0.59	0.11
Milk, kg/d	36.6	35.1	35.3	1.81	0.02	0.78
ECM, ³ kg/d	36.3	34.9	33.8	1.89	0.19	0.35
ECM/DMI	1.59	1.52	1.52	0.04	0.13	0.98
Milk fat, %	3.22	3.36	3.10	0.17	0.45	0.17
Milk protein, %	2.94	3.03	2.90	0.05	0.04	0.01
Milk lactose, %	4.83	4.81	4.80	0.07	0.57	0.99
Milk energy, ⁴ Mcal/kg	0.67	0.68	0.66	0.01	0.52	0.21
Milk fat, kg/d	1.21	1.19	1.10	0.09	0.77	0.27
Milk protein, kg/d	1.10	1.06	1.05	0.06	0.14	0.59
Milk lactose, kg/d	1.82	1.69	1.73	0.05	0.01	0.24
Milk energy, ⁴ Mcal/d	24.7	23.7	23.0	1.21	0.18	0.38
MUN, ⁵ mg/dL	12.8	10.2	10.9	0.74	0.01	0.10

¹109MP = high phase OSC diet fed continuously; 95MP = 50:50 mix of high and low phase OSC diets fed continuously; OSC = oscillating from the high phase OSC diet to low phase OSC diet on a 24-h basis. All LSM were covariate adjusted.

²CP concentration: 109MP vs. 95MP, positive control (16% CP) vs. negative CP control (14% CP); oscillation: 95MP vs. OSC, negative control (14% CP) vs. oscillating CP (14% CP).

³ECM, kg/d = $0.327 \times \text{milk yield} + 12.95 \times \text{fat yield} + 7.65 \times \text{protein yield}$ (Tyrrell and Reid, 1965).

⁴Calculated according to NRC (2001).

⁵Treatment $\times$ week interaction ($P < 0.01$).

Table 5. Effects of static or oscillating CP concentrations on day-to-day differences in intake, milk production, and blood metabolites¹

Item	109MP		95MP		OSC		SEM	T × P	P-value ²		
	High	Low	High	Low	High	Low			109MP	95MP	OSC
Production											
DMI, kg/d	23.0	22.9	23.2	23.0	22.6	22.1	0.97	0.83	0.86	0.97	0.01
Milk, kg/d	37.0	37.1	35.3	35.3	34.8	36.3	1.37	0.10			
ECM, kg/d	35.5	36.2	36.7	36.1	32.2	33.0	1.47	0.63			
Milk fat, kg/d	1.16	1.20	1.31	1.26	1.02	1.02	0.07	0.75			
Milk protein, kg/d	1.10	1.11	1.08	1.09	1.00	1.06	0.04	0.10	0.57	0.79	0.04
Milk lactose, kg/d	1.83	1.84	1.69	1.70	1.68	1.74	0.05	0.13			
MUN, mg/dL	12.2	12.5	10.3	10.7	9.0	12.4	0.50	0.01	0.68	0.43	0.01
Plasma metabolite ³											
Albumin, g/dL	5.33	5.54	5.30	5.33	5.33	5.56	0.09	0.58			
Globulin, ⁴ g/dL	2.95	2.83	2.85	2.78	2.86	2.62	0.20	0.76			
Total protein, g/dL	8.29	8.37	8.18	8.14	8.17	8.06	0.20	0.60			
FA, μ Eq/mL	114.4	112.3	107.5	103.6	85.4	82.7	18.1	0.13			
Glucose, mg/dL	69.6	70.5	68.5	69.6	67.4	69.4	1.48	0.82			
Insulin, μ U/mL	14.5	16.9	11.7	13.4	13.7	15.0	2.15	0.93			
EAA, μ mol/L	992	996	813	795	813	765	54.7	0.88			
NEAA, μ mol/L	1,276	1,263	1,266	1,247	1,276	1,249	38.1	0.98			
3-Methyl-His, μ mol/L	4.97	5.03	5.32	5.31	5.20	5.13	0.40	0.98			

¹Averages of high or low day of OSC within a treatment and for the 50-d treatment period. Day of OSC is whether cows on the OSC treatment were on the high phase OSC diet or low phase OSC diet. All LSM were covariate adjusted to measurements taken before treatment feeding except for plasma AA. 109MP = high phase OSC diet fed continuously; 95MP = 50:50 mix of high and low phase OSC diets fed continuously; OSC = oscillating from the high phase OSC diet to low phase OSC diet on a 24-h basis.

²The interaction of treatment and oscillation phase (T × P). If the interaction was significant ($P < 0.10$), then the SLICE option (SAS version 9.4, SAS Institute Inc., Cary, NC) was used to determine whether response variables differed across phases within a dietary treatment. The P -values under treatment columns are to evaluate differences between phases.

³Plasma metabolites are from samples taken on consecutive mornings during the middle (group 1: d 24 and 25; group 2: d 27 and 28) and end of treatment feeding (d 49 and 50). For plasma AA, samples were taken at the end of treatment feeding (d 49 and 50). FA = fatty acids.

⁴Globulin, g/dL = total protein, g/dL – albumin, g/dL.

centration was greater for cows fed 109MP and lowest for cows fed 95MP; the lack of a discernable pattern suggests the week by treatment interaction is probably not important biologically. Concentration of MUN was about 2.6 mg/dL greater ($P < 0.01$) for cows fed 109MP compared with 95MP, which agrees closely with values obtained from the prediction equation of Jonker et al. (1998). The MUN concentration also increased numerically ($P = 0.10$) for OSC compared with 95MP. Higher MUN concentration occurred despite similar milk and milk protein yields and less N intake for OSC compared with 95MP. The MUN concentration of cows fed OSC was also higher during the low phase compared with high phase diet (Table 5). Because MUN concentration is highly correlated with blood urea concentration (Broderick and Clayton, 1997), urea recycling to the rumen may have been increased when cows were fed the low phase diet (Doranalli et al., 2011), but the urea was not well utilized for microbial CP synthesis.

Plasma Metabolite and AA Concentrations

Plasma concentrations and indices of energy and protein metabolites were similar between 109MP and 95MP and for 95MP and OSC (Table 8). Low protein

diets (12.7 vs. 16.6% CP) have reduced plasma albumin but not total protein concentrations in mid-lactation cows (Raggio et al., 2007). In our study the 95MP or OSC were only 5% deficient in MP compared with over 20% deficiency in Raggio et al. (2007), which may be the reason we did not observe a treatment effect on plasma proteins. Plasma concentrations of fatty acids, glucose, and insulin as well as indices of insulin resistance (i.e., HOMA-IR) and sensitivity (i.e., RQUICKI) were unaffected likely because energy balance was positive and similar among treatments. Lack of treatment effects on plasma energy metabolites and indices support the lack of differences in change in body composition (discussed below, Table 9), BCS, BW, and milk energy yields. These data in total indicate that partitioning of NE_L to the mammary gland and peripheral tissues was not altered by treatments. Plasma metabolites did not differ ($P \geq 0.13$) between high and low days for the OSC treatment, although some N metabolites (e.g., total protein and AA) appear numerically lower on low versus high days of OSC (Table 5), which was opposite of what was observed for milk production.

Plasma concentrations of several EAA (Arg, His, Iso, Leu, Lys, and Val; Table 7) and NEAA (Ala, Glu, Gln, and Pro) were less ($P \leq 0.05$) for 95MP than for

Table 6. Effects of static or oscillating CP concentrations on manure output, DM and water intake, and nutrient digestibility

Item	Treatment ¹			SEM	P-value ²	
	109MP	95MP	OSC		109MP vs. 95MP	95MP vs. OSC
Water intake, kg/d						
Dietary	27.5	28.4	26.5	1.00	0.46	0.11
Free	108.8	100.0	94.4	5.10	0.09	0.24
Total	136.4	128.5	120.9	5.70	0.15	0.15
Output, kg/d (as-is)						
Milk	36.6	35.5	35.0	1.21	0.36	0.70
Feces	47.4	51.0	47.2	2.68	0.14	0.11
Fecal water	40.9	43.8	40.7	2.45	0.18	0.13
Urine	25.2	17.8	19.4	2.81	0.07	0.67
Total manure	72.9	68.8	66.6	3.28	0.30	0.56
DM intake, kg/d	21.5	22.6	20.9	0.74	0.16	0.03
Apparent digestibility, ³ %						
DM	69.5	68.1	68.5	0.78	0.19	0.70
OM	70.7	69.0	69.4	0.79	0.14	0.72
CP	65.2	61.7	65.3	1.44	0.09	0.07
NDF	54.1	53.5	52.7	1.97	0.82	0.77
NDF _{om+cp} ⁴	54.3	53.3	52.6	2.07	0.72	0.82
Starch	94.2	92.8	93.6	0.76	0.12	0.35
LCFA	71.8	69.9	71.2	1.64	0.39	0.55
ROM	71.1	66.5	65.3	2.50	0.16	0.69
ROM _{om+cp} ⁴	69.0	65.5	64.1	2.12	0.21	0.58

¹109MP = high phase OSC diet fed continuously; 95MP = 50:50 mix of high and low phase OSC diets fed continuously; OSC = oscillating from the high phase OSC diet to low phase OSC diet on a 24-h basis.

²CP concentration: 109MP vs. 95MP, positive control (16% CP) vs. negative CP control (14% CP); oscillation: 95MP vs. OSC, negative control (14% CP) vs. oscillating CP (14% CP).

³LCFA = long-chain fatty acid; ROM = residual OM.

⁴NDF_{om+cp} = NDF – NDI-ash (ashed NDF residues) – NDICP (NDF residues analyzed for CP); ROM_{om+cp} = 100 – %CP – %LCFA – %ash – %starch – %NDF_{om+cp}.

109MP. Citrulline and ornithine, intermediate AA in the urea cycle, were also lower ($P \leq 0.05$) for 95MP compared with 109MP. Decreases in these plasma AA with a lower MP supply are in line with other studies (Lee et al., 2012; Giallongo et al., 2015). For OSC treatment, dietary CP changed markedly day to day, but plasma concentrations of most AA were remarkably similar over the oscillation cycle (Table 5, Supplemental Table S3; <https://doi.org/10.3168/jds.2020-18613>). To our knowledge, no previous studies oscillating CP concentrations in ruminants have measured AA concentrations or day-to-day changes of AA concentrations in plasma. Except for Arg and Thr, average plasma concentrations across high and low days of OSC were similar for all AA ($P \geq 0.15$) between 95 MP and OSC. Arginine can be endogenously synthesized in the intestine, is essential for the urea cycle, and can serve as an N reservoir (Wu, 2009). Plasma Arg numerically increased ($P = 0.11$) for OSC compared with 95MP, but the reason is unknown. Conversely, Thr tended to decrease ($P = 0.09$) when oscillating CP. In addition to milk protein, Thr is used largely to synthesize mucin proteins required to maintain gut integrity and function (Wu, 2009). However, Thr is likely not a limiting AA for milk protein synthesis (Doepel et al., 2016). Plasma

concentrations of 3-methyl-L-His, a biomarker for muscle catabolism, were similar between OSC and 95MP indicating endogenous stores (i.e., skeletal muscle) were probably not supplying extra AA for milk production when oscillating CP. Moreover, plasma 3-methyl-His concentrations were similar between high and low days of oscillation for OSC (Table 5). The lack of differences in 3-methyl-His provides additional evidence that protein stores cannot rapidly buffer a temporary reduction in MP supply.

Nutrient Intake and Apparent Digestibility

During the 4-d total collection period (Table 6), DMI was similar between 109MP and 95MP but about 0.7 kg/d less ($P < 0.03$) for OSC compared with 95MP. Milk yield during the digestion trial followed the same pattern as during the production experiment (Table 4), but because of less statistical power and the short duration (4 d) no statistical differences were observed.

Free water intake and urine output tended to increase ($P \leq 0.09$) for 109MP versus 95MP. This agrees with other findings where dietary CP concentrations have been positively correlated with free water intake (Holter and Urban, 1992) and urine output (Bannink

Table 7. Effects of static or oscillating CP concentrations on average plasma AA concentrations¹

Item, $\mu\text{mol/L}$	Treatment ²			SEM	P-value ³	
	109MP	95MP	OSC		109MP vs. 95MP	95MP vs. OSC
AA						
Arginine	77.1	57.7	65.2	3.34	0.01	0.11
Histidine	52.5	33.9	31.2	5.46	0.01	0.42
Isoleucine	152	119	114	8.13	0.01	0.58
Leucine	122.9	96.5	96.9	6.52	0.01	0.95
Lysine	69.2	53.9	54.2	3.94	0.01	0.96
Methionine	18.7	17.4	17.3	1.08	0.23	0.93
Phenylalanine	36.0	32.8	34.0	1.58	0.14	0.58
Threonine	89.8	87.9	80.2	4.04	0.68	0.09
Tryptophan	72.4	66.9	58.8	5.55	0.34	0.16
Valine	305	238	239	13.9	0.01	0.96
EAA	994	804	789	37.4	0.01	0.76
Alanine	293	266	263	9.49	0.03	0.79
Aspartate	5.8	5.6	5.2	0.29	0.49	0.38
Asparagine	54.9	55.1	53.5	4.19	0.98	0.78
Glutamate	63.2	56.6	59.8	3.78	0.05	0.34
Glutamine	255	226	243	13.1	0.03	0.16
Glycine	338	404	396	21.9	0.01	0.57
Proline	123	112	111	3.95	0.06	0.82
Serine	88.8	97.3	102.1	4.86	0.08	0.30
Tyrosine	37.5	34.1	31.3	1.71	0.15	0.24
NEAA	1,270	1,257	1,263	26.6	0.69	0.86
1-Methyl-L-histidine	4.1	4.5	4.7	0.24	0.11	0.25
3-Methyl-L-histidine	5.0	5.3	5.2	0.30	0.33	0.65
Citrulline	105.6	96.7	99.3	3.06	0.04	0.54
Ornithine	61.8	47.8	51.2	3.73	0.01	0.51

¹Plasma AA were from samples taken at the end of treatment feeding (d 49 and 50). Averages calculated across the consecutive days of sampling.

²109MP = high phase OSC diet fed continuously; 95MP = 50:50 mix of high and low phase OSC diets fed continuously; OSC = oscillating from the high phase OSC diet to low phase OSC diet on a 24-h basis.

³CP concentration: 109MP vs. 95MP, positive control (16% CP) vs. negative CP control (14% CP); oscillation: 95MP vs. OSC, negative control (14% CP) vs. oscillating CP (14% CP).

Table 8. Effects of static or oscillating CP concentrations on the plasma concentration of energy- and protein-related metabolites¹

Item	Treatment ²			SEM	P-value ³	
	109MP	95MP	OSC		109MP vs. 95MP	95MP vs. OSC
Albumin, g/dL	5.44	5.32	5.40	0.06	0.15	0.34
Globulin, ⁴ g/dL	2.89	2.82	2.74	0.18	0.72	0.72
Total protein, g/dL	8.33	8.16	8.12	0.19	0.41	0.87
FA, ⁵ $\mu\text{Eq/mL}$	98.4	105.6	84.1	17.1	0.64	0.20
Glucose, mg/dL	70.1	69.1	68.4	1.32	0.58	0.71
Insulin, $\mu\text{U/mL}$	15.7	12.5	14.4	1.92	0.21	0.48
HOMA-IR ⁶	2.76	2.15	2.50	0.35	0.20	0.46
RQUICKI ⁷	0.204	0.206	0.210	0.004	0.65	0.48

¹Plasma metabolites are from samples taken on consecutive mornings during the middle (group 1: d 24 and 25; group 2: d 27 and 28) and end of treatment feeding (d 49 and 50). All LSM were covariate adjusted to plasma samples taken before treatment feeding.

²109MP = high phase OSC diet fed continuously; 95MP = 50:50 mix of high and low phase OSC diets fed continuously; OSC = oscillating from the high phase OSC diet to low phase OSC diet on a 24-h basis.

³CP concentration: 109MP vs. 95MP, positive control (16% CP) vs. negative CP control (14% CP); oscillation: 95MP vs. OSC, negative control (14% CP) vs. oscillating CP (14% CP).

⁴Globulin, g/dL = total protein, g/dL – albumin, g/dL.

⁵FA = fatty acids.

⁶Homeostasis model assessment of insulin resistance (HOMA-IR) = [glucose (mg/dL) $\times$ insulin ($\mu\text{U/mL}$)]/405. Higher HOMA-IR means greater insulin resistance.

⁷Revised quantitative insulin sensitivity check index (RQUICKI) = [log glucose (mg/dL) + log insulin ($\mu\text{U/mL}$) + log FA ($\mu\text{Eq/L}$)]⁻¹ Lower RQUICKI means greater insulin sensitivity.

Table 9. Effects of static or oscillating CP concentrations on body composition measured using urea dilution method¹

Item	Treatment ²			SEM	P-value ³	
	109MP	95MP	OSC		109MP vs. 95MP	95MP vs. OSC
BCS	3.18	3.20	3.24	0.08	0.85	0.66
Δ, score/wk*	0.03	0.04	0.04	0.01	0.71	0.72
BW, kg	687	686	687	1.2	0.23	0.67
Δ, kg/wk*	5.8	4.7	4.3	2.00	0.48	0.77
Empty BW, kg	487	487	491	6.7	0.95	0.68
Δ, kg/wk	1.11	1.03	1.59	0.93	0.95	0.67
Water, kg	303	302	304	4.5	0.86	0.68
Δ, kg/wk	-0.28	-0.41	-0.02	0.64	0.89	0.67
Lipid, kg	84.2	84.5	84.7	1.67	0.89	0.91
Δ, kg/wk*	1.70	1.78	1.85	0.26	0.78	0.81
CP, kg	88.2	87.8	88.7	1.46	0.87	0.68
Δ, kg/wk	-0.09	-0.13	-0.01	0.21	0.90	0.66
Ash, kg	27.9	27.8	28.2	0.71	0.92	0.66
Δ, kg/wk	-0.07	-0.08	-0.01	0.10	0.94	0.64
Gross energy, Mcal	1,318	1,318	1,321	19.4	0.98	0.93
Δ, Mcal/wk*	17.8	18.3	19.0	2.94	0.88	0.82

¹Body composition measurements are calculated using the equations in Agnew et al. (2005) and covariate adjusted to measurements taken before treatment feeding. Change (Δ) in body composition is unadjusted and is calculated as the difference between measurements taken before and after treatment feeding.

²109MP = high phase OSC diet fed continuously; 95MP = 50:50 mix of high and low phase OSC diets fed continuously; OSC = oscillating from the high phase OSC diet to low phase OSC diet on a 24-h basis.

³CP concentration: 109MP vs. 95MP, positive control (16% CP) vs. negative CP control (14% CP); oscillation: 95MP vs. OSC, negative control (14% CP) vs. oscillating CP (14% CP).

*Means within a row were all different from 0 ($P < 0.05$).

et al., 1999). Total water intake and output of manure were not affected by MP or oscillation ($P > 0.15$).

Intakes of OM, LCFA, and ROM were similar between 109MP and 95MP, whereas intakes of CP, starch, and NDF reflected changes in their dietary concentrations between treatments (data not shown). Digestibility of most nutrients was similar between 109MP and 95MP with the exception of CP, which was decreased when lowering the CP concentration. Apparent CP digestibility usually increases when dietary CP concentration increases because metabolic fecal CP is diluted (Holter and Reid, 1959). Low CP (<15% of DM) and RDP (<10% of DM) concentrations can reduce NDF digestibility (Broderick et al., 2008; Lee et al., 2011). Similar NDF digestion suggests RDP may not have been deficient for 95MP or more urea was recycled to the rumen for 95MP versus 109MP.

Average nutrient concentrations between 95MP and OSC were similar; however, the decrease in DMI for OSC caused intake of major nutrients to decrease ($P \leq 0.04$) around 8% compared with 95MP during the total collection period (data not shown). Except for CP, digestibility of major nutrients was similar between 95MP and OSC. The digestibility of CP increased from 61.7 to 65.3% when oscillating CP concentrations. In previous studies, digestibility of DM and different carbohydrate fractions was similar whether CP concentrations were oscillated or static; however, oscillating CP on a 48-h

basis versus feeding a similar average CP concentration continuously has increased (Archibeque et al., 2007a; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011), not affected (Cole, 1999; Ludden et al., 2002b; Cole et al., 2003; Menezes et al., 2019), or decreased CP digestibility (Ludden et al., 2002a). Dissimilar results are likely from the wide range of diets fed and different manipulations of other nutrients when oscillating CP (i.e., starch, NDF, RDP, RUP). In studies that observed greater CP digestibility, protein sources with moderate to low ruminal CP degradability (e.g., canola, soybean, or corn gluten meal) were oscillated with starch sources (e.g., ground corn or barley). In studies with similar CP digestibility, protein sources with moderate to low ruminal CP degradability and urea were oscillated with starch sources (e.g., ground corn or sorghum). In the only study with lower CP digestibility, a protein source high in RUP (i.e., expeller soybean meal) was oscillated with soybean meal, urea, and corn in low CP diets to make RDP equal. Our results partially agree with studies with increased CP digestibility since we oscillated soybean meal with corn grain and soyhulls and did not balance for RDP. Therefore, higher CP digestion may be observed when oscillating primarily RDP concentrations and highly digestible carbohydrates. More urea recycling to the rumen versus hindgut when oscillating CP concentrations as suggested by Cole (1999) could explain greater apparent digestibility of CP. Because

Table 10. Effects of static or oscillating CP concentrations on estimated dietary energy intakes and losses

Item	Treatment ¹			SEM	P-value ²	
	109MP	95MP	OSC		109MP vs. 95MP	95MP vs. OSC
DE intake, ³ Mcal/d	65.6	66.7	62.4	2.48	0.69	0.12
DE, ³ Mcal/kg of DM	3.05	2.95	2.97	0.04	0.06	0.59
Urine energy, ⁴ Mcal/d	2.1	1.7	2.0	0.13	0.05	0.15
Methane energy, ⁵ Mcal/d	6.0	6.4	5.9	0.22	0.11	0.04
NE _L , ⁶ Mcal/kg of DM	1.76	1.71	1.72	0.02	0.10	0.85
NE _L , ⁷ Mcal/kg of DM	1.76	1.74	1.69	0.05	0.55	0.24

¹109MP = high phase OSC diet fed continuously; 95MP = 50:50 mix of high and low phase OSC diets fed continuously; OSC = oscillating from the high phase OSC diet to low phase OSC diet on a 24-h basis.

²CP concentration: 109MP vs. 95MP, positive control (16% CP) vs. negative CP control (14% CP); oscillation: 95MP vs. OSC, negative control (14% CP) vs. oscillating CP (14% CP).

³Digestible energy (DE) intake (Mcal/d) calculated according to Tebbe et al. (2018).

⁴Estimated urine energy, Mcal/d = $0.033 \times \text{urine C, g/d} + 0.009 \times \text{urine N, g/d}$ from Hoffmann and Klein (1980).

⁵Predicted methane energy, Mcal/d = $\text{DMI, kg/d} \times 0.294 - 0.347 \times \text{long-chain fatty acids, \% of DM} + 0.0287 \times \text{NDF, \% of DM}$ from Nielsen et al. (2013).

⁶NE_L, Mcal/kg = $[0.66 \times (\text{DE intake} - \text{urine energy} - \text{methane energy})]/\text{DMI}$.

⁷NE_L, Mcal/kg = $(0.1 \times \text{BW}^{0.75} + \text{BW change} \times 5.6 + \text{milk energy})/\text{DMI}$. Calculated using energy outputs measured during treatment feeding. Maintenance energy is calculated according to Ellis et al. (2006) and BW and milk energies according to NRC (2001).

most metabolic fecal CP originates from bacteria, a reduction of urea recycled to the hindgut and less hindgut microbial CP synthesis could explain increased apparent CP digestibility when oscillating CP. The proposed mechanism as well as the degradability of CP sources and nutrients replacing CP when oscillating deserves more investigation.

Energy and N Metabolism

Gross feed efficiency was unaffected and averaged 1.54 kg of ECM/kg of DMI across treatments. Intake of estimated DE was similar between 109MP and 95MP and averaged 66.2 Mcal/d (Table 10). Increasing dietary CP concentration often increases DM digestibility (Oldham, 1984) and presumably energy digestibility. Numerically less DMI for 109MP caused similar DE intakes because estimated DE concentration tended to be 0.1 Mcal/kg greater ($P = 0.10$) for 109MP versus 95MP. Increasing the CP concentration increased ($P = 0.05$) estimated urinary energy but numerically decreased ($P = 0.11$) estimated methane losses. When assuming a constant efficiency of 0.66 Mcal of NE_L/Mcal of ME, this resulted in a tendency for greater ($P = 0.10$) estimated NE_L concentration for 109MP compared with 95MP (Table 10). However, because diets with higher CP concentration may reduce the efficiency of converting ME to NE_L (Reed et al., 2017; Morris et al., 2019b), our calculations may overestimate the NE_L concentration for the 109MP diet relative to the 95MP treatments. When diet NE_L was estimated from energy expenditures, dietary values were similar to those obtained from the method based on DE. In

steers, oscillating CP compared with a similar average CP concentration fed continuously tended to reduce heat production 4% without affecting other energetic losses (Archibeque et al., 2007a). If this happened in our study, the OSC treatment would have reduced heat losses and improved efficiency of ME to NE_L compared with 95MP, and the estimated NE_L for the OSC treatment would be underestimated relative to the 95MP treatment.

The greater mass of N absorbed for 109MP did not improve productive-N output ($P = 0.40$; Table 11) and instead increased urinary N excretion by 60 g/d compared with 95MP. Urinary output of creatinine, a biomarker for muscle mass, and purine derivatives, indicators of microbial CP synthesis, were similar ($P \geq 0.31$) for 109MP and 95MP. These results support our contention that N in the 109MP diet was in excess of requirements for the cow and rumen microbes.

When oscillating CP, dietary N intake was reduced 38 g/d compared with 95MP; however, cows fed OSC had similar intakes of digestible N as 95MP. Greater N digestibility for OSC did not improve NUE and MP supply compared with 95MP because milk N tended to be less and retained N was numerically less for OSC. Rather, greater N digestibility led to more N being excreted in urine. Excretion of purine derivatives was similar between 95MP and OSC. This disagrees with Doranalli et al. (2011) who found oscillating CP in growing lambs decreased urine N excretion and increased urinary output of purine derivatives. In our study, oscillating CP may have increased urea recycling to the rumen, but less DE intake may have limited the ability of rumen microbes to use the recycled N (Nocek

and Russell, 1988) and failed to increase NUE when oscillating CP. Retained N did not differ from zero ($P = 0.57$), which agrees with the body composition data (Table 9), and there was a tendency for greater ($P = 0.08$) creatinine excretion for OSC ($P = 0.08$). These results give further evidence that the catabolism of endogenous N is probably not the major mechanism causing increased N excretion.

Body Composition

Body composition can be predicted accurately with the urea dilution technique (Agnew et al., 2005) and this can be repeated on the same animal. Repeated measurements allow changes in body composition to be calculated. Average estimates of empty body water (mean $\pm$ SD; 62.2 ± 1.93), lipid (17.1 ± 2.15), protein (18.1 ± 0.48), and ash (5.7 ± 0.32) as a percentage of empty BW (Table 9) were similar to slaughter studies in dairy cows (Gibb et al., 1992; Andrew et al., 1994; von Soosten et al., 2012) and to predicted values for cows with a BCS of 3.2 (NRC, 2001).

In our study, feeding different concentrations of CP or oscillating CP for 50 d had minimal effects on the final and weekly changes of BCS, BW, empty BW, and empty body components ($P > 0.15$). The weekly change in BW, and empty body lipid and gross en-

ergy were greater than zero for all treatments ($P < 0.05$), confirming cows were in positive energy balance. Weekly changes in empty body CP were not different ($P \geq 0.51$) from zero for any treatments, indicating these cows did not accrete or lose empty body protein during the 50-d feeding period. Body composition measurements, N balance data, and plasma 3-methyl-His concentrations indicate that over the 50-d experiment cows fed 95MP or OSC CP were not mobilizing protein stores.

CONCLUSIONS

Compared with feeding a similar average CP concentration continuously, oscillating the CP concentrations of a diet 5% deficient in MP on a 24-h basis reduced DMI but not milk production. Oscillating CP decreased the proportion of consumed N that was excreted in feces but increased urinary N, suggesting some other factor, such as reduced intake of DE, was limiting the ability of the cow to convert digested protein into milk. Daily milk and milk protein yields responded to the daily changes in dietary protein, indicating that in mid lactation cows cannot buffer short-term protein deficiencies from body reserves. Concentrations of plasma 3-methyl-His and changes in body protein stores support that suggestion. Future work oscillat-

Table 11. Effects of static or oscillating CP concentrations on N metabolism and excretion of urine metabolites

Item	Treatment ¹			SEM	P-value ²	
	109MP	95MP	OSC		109MP vs. 95MP	95MP vs. OSC
N metabolism						
Intake, g/d	561	512	474	16.9	0.01	0.03
Digested (ND), g/d	366	317	310	14.2	0.01	0.66
Milk, g/d	174	179	164	5.63	0.50	0.06
Urinary, g/d	185	124	151	12.6	0.01	0.13
Fecal, g/d	194	196	165	9.06	0.88	0.02
Retention, g/d	5.5	14.2	-5.4	10.0	0.49	0.11
Productive N, g/d	179	193	159	12.3	0.40	0.04
Milk, % of intake	31.2	34.9	35.1	1.21	0.04	0.90
Urinary, % of intake	33.3	24.4	31.6	2.37	0.01	0.03
Productive N, ³ % of intake	32.0	37.3	33.7	1.83	0.05	0.15
Milk, % of ND	48.1	56.7	53.7	2.37	0.02	0.35
Urinary, % of ND	50.5	39.5	48.4	3.10	0.02	0.04
Productive N, ³ % of ND	49.5	60.5	51.6	3.09	0.02	0.04
Urinary excretion, mmol/d						
Creatinine	124	111	133	9.1	0.31	0.08
Allantoin	305	269	281	27.6	0.35	0.75
Uric acid	28.5	31.3	27.7	4.82	0.68	0.58
Total PD ⁴	333	300	309	30.2	0.43	0.84
Total PD/kg of OM digested	23.5	20.8	22.9	2.18	0.38	0.48

¹109MP = high phase OSC diet fed continuously; 95MP = 50:50 mix of high and low phase OSC diets fed continuously; OSC = oscillating from the high phase OSC diet to low phase OSC diet on a 24-h basis.

²CP concentration: 109MP vs. 95MP, positive control (16% CP) vs. negative CP control (14% CP); oscillation: 95MP vs. OSC, negative control (14% CP) vs. oscillating CP (14% CP).

³Productive N, g/d = milk N, g/d + retention, g/d.

⁴Total purine derivatives (PD), mmol/d = allantoin, mmol/d + uric acid, mmol/d.

ing CP to dairy cows should evaluate the type of CP sources substituted (i.e., ruminal degradability of CP) and what nutrients replace CP in the deficient CP diet to minimize negative effects on DMI.

ACKNOWLEDGMENTS

The authors express appreciation to Donna Wyatt (Ohio State University, Wooster) for laboratory analyses, and to Brent Hostettler and his crew at the Ohio State University Krauss Dairy Center. This project was supported in part by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, Wooster, OH. The authors have not stated any conflicts of interest.

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