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Concurrent and carryover effects of feeding blends of protein and amino acids in high-protein diets with different concentrations of forage fiber to fresh cows. 2. Protein balance and body composition

A. W. Tebbe* and W. P. Weiss†
Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691

ABSTRACT

Increasing the supply of metabolizable protein (MP) and improving its AA profile may attenuate body protein mobilization in fresh cows and lead to increased milk production. Increasing the concentration of rumen-undegradable protein (RUP) to increase MP supply and replacing RUP sources from forages instead of nonforage fiber sources may further decrease tissue mobilization if it improves dry matter intake (DMI). Our objective was to determine whether increasing MP concentrations and improving the AA profile at the expense of either nonforage or forage fiber (fNDF) would affect MP balance and empty body (EB) composition (measured using the urea dilution method) in early postpartum dairy cows of different parities. In a randomized block design, 40 primigravid [77 ± 1.5 kg of EB crude protein (CP) at 8 ± 0.6 d before calving] and 40 multigravid (92 ± 1.6 kg of EB CP at 5 ± 0.6 d before calving) Holsteins were blocked by calving date and fed a common prepartum diet (11.5% CP). After calving to 25 d in milk (DIM), cows were fed 1 of 4 diets: (1) a diet deficient in MP meeting 87% of MP requirements (DMP, 17% CP, 24% fNDF), (2) 104% of MP requirements using primarily soy protein to make MP adequate (AMP, 20% CP, 24% fNDF), (3) 110% of MP requirements using a blend of proteins and rumen-protected (RP) AA to make MP adequate (Blend, 20% CP, 24% fNDF), or (4) a diet similar to Blend but substituting added RUP for fNDF rather than nonforage NDF (Blend-fNDF, 20% CP, 19% fNDF). Blend was formulated to have a RUP supply with a similar AA profile to that of casein. Cows were fed a common diet (16.3% CP) from 26 to 50 DIM. Calculated MP balance (supply − requirements) was less than zero for DMP and Blend-fNDF from 1 to 4 wk of lactation (WOL), whereas that for AMP was positive from 1 to 4 WOL and that for Blend was close to zero from 3 to 4 WOL. Daily MP balance was greater from 5 to 7 WOL for DMP compared with AMP and Blend (100 vs. 22 g/d). From −7 to 7 d relative to calving, losses of EB CP were greater for DMP than for AMP and Blend (−121 vs. average of 11 g/d). From 7 to 25 DIM, cows fed AMP (−139 g/d) and Blend-fNDF (−147 g/d) lost EB CP but cows fed Blend (−8 g/d) maintained EB CP. Increased DMI for Blend versus AMP led to reduced losses of EB lipid in primiparous cows from 7 to 25 d relative to calving (−1.0 vs. −1.3 kg/d of EB lipid), whereas lipid mobilization was similar in multiparous cows (average −1.1 kg of EB lipid/d). By 50 DIM, EB lipid and CP were similar across treatments and parities (average 60.2 kg of EB lipid and 81.6 kg of EB CP). Overall, feeding fresh cows a high MP diet with a balanced AA profile improved DMI and attenuated EB CP mobilization, which could partly explain positive carryover effects on milk production for multiparous cows and reduced lipid mobilization for primiparous cows.

Key words: forage neutral detergent fiber (fNDF), parity, body composition, body protein and lipid

INTRODUCTION

In fresh cows, low DMI causes low nutrient intakes and can cause deficiencies of NE_L and MP, which induce mobilization of body lipid and protein and may negatively influence milk production (Grummer, 1995; Bell et al., 2000). In contrast to increasing diet NE_L concentrations, increasing MP concentrations often improves milk production because fresh cows have a greater capacity to mobilize lipid versus protein to meet lactational demands (Schei et al., 2005). It is unclear whether improved milk production with greater MP supply is mediated by less protein mobilization.

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*Current address: Purina Animal Nutrition LLC, Shoreview, MN 55126.
†Corresponding author: weiss.6@osu.edu

Cows fed diets with increased MP concentration (10.6 vs. 12% of DM) or a diet with a 12% MP and balanced for Lys and Met from 3 to 21 DIM reduced plasma 3-methyl-histidine concentrations, a marker of skeletal muscle breakdown, suggesting less protein mobilization (Carder and Weiss, 2017). Increasing MP from 10.1 to 13.7% of DM from 1 to 21 DIM also increased calculated MP balance (Amanlou et al., 2017). However, feeding 19% versus 16% CP did not reduce estimated losses of empty body (**EB**) CP from 2 wk before parturition to 5 or 12 wk of lactation (**WOL**; Komaragiri and Erdman, 1997). Gradually decreasing infusions of casein in the abomasum from 720 g/d at 2 DIM to 194 g/d by 29 DIM led to greater MP balance at 4 DIM, but MP balance was similar at 15 DIM and lower by 29 DIM for cows infused with casein compared with water (Larsen et al., 2014).

Inconsistent effects of altering MP supply on MP balance may result from the variable effects on DMI in fresh cows in these studies: increased (Amanlou et al., 2017), unchanged (Carder and Weiss, 2017; Larsen et al., 2014), and decreased (Komaragiri and Erdman, 1997). Improving the AA supply when feeding an MP-deficient diet increased DMI in mid-lactation cows (Giallongo et al., 2016). What replaces the CP when increasing MP concentration could also affect DMI. Replacing forage NDF (**fNDF**) for nonforage NDF can increase DMI (Allen, 2000). Increasing CP concentration at the expense of forages rather than concentrates could increase DMI in fresh cows because DMI may be limited by physical fill after calving (Piantoni et al., 2015).

The objectives were to determine whether feeding high MP diets using a single source of protein or a blend of proteins high in RUP and AA sources to improve AA balance and whether substituting fNDF rather than nonforage NDF with RUP would affect EB composition and MP balances. We also wanted to determine whether these effects carried over after treatments ceased. Because body growth affects MP requirements and partitioning, differential effects between primiparous and multiparous cows were also evaluated.

MATERIALS AND METHODS

Experimental Design and Treatments

All procedures involving animals were approved by The Ohio State University Institutional Animal Care and Use Committee (Protocol #2018A00000093). Forty primigravid and 40 multigravid cows were used in a randomized block experiment. Cows were blocked in groups of 4 based on parity and expected calving date. The experiment consisted of 3 phases: prepartum, treatment, and carryover. During the prepartum phase, cows were moved to individual box stalls 14 d before anticipated calving and fed a common, close-up dry cow diet (11.5% CP, 56% NDF, 1.37 Mcal/kg). Immediately after calving, cows began the treatment phase and received 1 of 4 diets (Table 1): (1) a diet formulated (NRC, 2001) to be 20% deficient in MP (**DMP**), (2) a diet formulated to meet MP requirements using primarily lignosulfonate-treated soybean meal to increase RUP (adequate MP; **AMP**), (3) a diet formulated to meet MP requirements using a blend of feed ingredients high in RUP and rumen-protected (**RP**) AA (Blend), and (4) a diet similar to Blend but replacing a portion of fNDF rather than nonforage NDF with the blend of protein and AA (Blend-fNDF). The blend of RUP and RP AA sources was formulated to have a RUP supply with an AA profile similar to that of casein (Tebbe and Weiss, 2021). Fresh cows were moved to tiestalls between 2 and 4 DIM and continued treatment diets until 25 DIM. At 26 DIM, cows began the carryover phase and switched to a common, early lactation diet until 51 DIM (Table 1).

Treatment diets were formulated using NRC (2001) for cows at 10 DIM with an estimated DMI of 16 kg and estimated milk yield of 34 kg/d with 3.9% fat and 3.2% protein. Treatment diets were also formulated for similar and adequate RDP concentrations, to exceed mineral and vitamin requirements, and to have 9.6 g/d of supplemental metabolizable Met. The AMP, Blend, and Blend-fNDF diets were balanced for similar CP, RUP, and NDF concentrations but differing in either AA composition (Lys, Met, His, respectively; AMP: 6.2, 2.0, 2.2% of MP vs. Blend and Blend-fNDF: 6.6, 2.3, and 2.3% of MP) or fNDF concentration. The carryover diet had similar nutrient concentrations to DMP but did not contain RP Met. More details of the experimental design, diet formulation, and effects on production and blood metabolites are presented in the companion paper (Tebbe and Weiss, 2021).

Metabolizable Protein Measurements

Feed samples that were taken weekly and composited by month (Tebbe and Weiss, 2021) were dried and ground through a 1-mm screen (Wiley mill; Arthur H. Thomas Co., Philadelphia, PA), and then mixed to make composite TMR samples that mimicked the 4 treatments diets (n = 3 TMR samples/treatment). These samples were analyzed for RDP and digestible RUP using 16-h in situ incubations and the mobile bag procedure, respectively (Kononoff et al., 2007; Paz et al., 2014). The CP concentration in composite samples

Table 1. Concentration of major nutrients and protein fractions in diets fed during the treatment and carryover phases[1]

Nutrient	Treatment[2]				Carryover
	DMP	AMP	Blend	Blend-fNDF	
NDF, % of DM	32.4	30.9	31.1	30.9	32.3
Forage NDF, % of DM	24.3	24.4	24.3	19.6	23.7
CP, % of DM	16.9	20.2	19.9	19.7	16.3
NE_L,[3] Mcal/kg	1.63	1.68	1.64	1.66	1.64
NRC RDP,[3] % of CP	61.5	52.5	51.8	52.8	62.7
RDP,[4] % of CP	62.5	48.2	58.8	59.3	—
NRC RUP digestibility,[3] g/g	0.83	0.88	0.88	0.90	0.84
RUP digestibility,[4] g/g	0.88	0.92	0.88	0.88	—
NRC MP,[3] % of DM	11.3	14.4	14.2	14.4	11.2
MP,[5] % of DM	10.8	14.9	12.4	11.9	—
MP_{adj},[6] % of DM	9.5	13.6	11.1	10.7	9.6

[1]During the treatment phase, cows were switched to 1 of 4 treatment diets immediately after calving until 25 DIM. During the carryover phase, cows were fed a common, early lactation diet (i.e., carryover) from 25 to 50 DIM.

[2]Treatments were deficient MP (DMP; 16.9% CP), adequate MP using primarily soy to increase RUP (AMP; 20.2% CP), adequate MP using a blend of protein and AA sources to increase RUP (Blend; 19.9% CP), and Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; 19.8% CP).

[3]Predicted using NRC (2001) model and nutrient composition library and treatment-average DMI.

[4]Treatment RDP was determined from a 16-h in situ incubation and RUP digestibility from the mobile bag procedure (Kononoff et al., 2007; Paz et al., 2014).

[5]MP, % = (digestible microbial CP, g/d + digestible RUP, g/d) ÷ DMI, g/d × 100. Digestible microbial CP was calculated from NRC (2001) and using RDP from the 16-h in situ incubation. Digestible RUP calculated from RDP (RUP, % of DM = CP − RDP) and RUP digestibility (see footnote 4).

[6]MP calculated (see footnote 5) and adjusted (MP_{adj}) for endogenous urinary true protein (endogenous urinary true protein, g/d = 0.33 × BW; Lapierre et al., 2020).

and residue from mobile bag samples were determined using an elemental analyzer (N × 6.25; Flash 2000, Thermo Fisher Scientific, Waltham, MA) after drying (45°C for 48 h) and pulverizing (MM200; Retsch GmbH, Haan, Germany).

The RDP (% of CP) was estimated as the amount of CP disappearing after the 16-h in situ incubation (Paz et al., 2014). Concentration of RUP was calculated by difference (RUP, % of DM = CP − RDP). To calculate MP supplied from microbial CP, microbial CP yield was calculated as 130 g/kg of discounted TDN intake, true protein (**TP**) was 0.80 g/g of microbial CP, and microbial TP digestibility was 0.80 g/g of microbial TP (NRC, 2001). To calculate digestible RUP supply, the estimated RUP supply was multiplied by digestibility of RUP determined using the mobile bag technique. The MP dietary supply was then calculated as the sum of microbial MP and digestible RUP. Digestible endogenous CP was not included in MP supply calculations because the residues used to calculate digestible microbial CP and RUP were unadjusted for bacterial contamination. The NRC (2001) was used to estimate MP supply for the carryover period diet.

Intakes of MP calculated from the method above and requirements of MP using NRC (2001) were calculated for each week (1 to 7 WOL) using weekly average DMI, milk yield, and milk composition. Concentration of milk TP was assayed weekly using composite milk samples (a.m. and p.m.) and analyzed by DHI Cooperative Inc. (Columbus, OH; B2000 Infrared Analyzer, Bentley Instruments, Chaska MN). Weekly average milk TP yield was calculated using weekly average milk yield and assayed milk TP concentration. For maintenance requirements, BW measured at 15, 35, and 50 DIM was used for wk 2, 5, and 7, respectively, whereas average BW measurements from calving to 7 DIM (timing detailed below), from 15 and 25 DIM, from 25 and 35 DIM, and from 35 and 50 DIM were used for wk 1, 3, 4, and 6, respectively. Postpartum MP balances were calculated as intake minus maintenance and milk requirements; MP required for growth was not included in MP balance calculations.

Utilization efficiency of MP (**MPUE**) and TP requirements were estimated using equations from Lapierre et al. (2020). To estimate MPUE, secretions of net protein for maintenance were calculated as the sum of estimated scurf TP (scurf TP, g/d = 0.17 × $BW^{0.6}$) and metabolic fecal TP [metabolic fecal TP, g/d = (8.5 + 0.1 × NDF, % of DM) × DMI, kg/d], and weekly average milk TP yield. Because MPUE for endogenous urinary TP is equal to 1, it was not included in maintenance requirements and subtracted from MP intake (endogenous urinary TP, g/d = 0.33 × BW; Lapierre et al., 2020). Therefore, estimated MPUE was calculated as follows:

$$\text{Estimated MPUE } (\%) = \frac{\begin{bmatrix}\text{scurf and metabolic fecal TP} \\ \text{secretions } (\text{g/d}) + \text{milk TP yield } (\text{g/d})\end{bmatrix}}{\text{MP intake } (\text{g/d}) - \text{endogenous urinary TP } (\text{g/d})} \times 100. \quad [1]$$

Body Composition, Score, and Weight Measurements

At approximately −7, 7, 25, and 50 d relative to calving, body composition was estimated using the urea dilution technique (Agnew et al., 2005). Measurements were conducted weekly about 5 h after feeding on Mondays, Wednesdays and Fridays; measurement day could deviate ±1 d from the target. The urea dilution procedure is detailed in Tebbe and Weiss (2020). Alternate sides of the cow's neck were used at successive time points. For each urea dilution, a baseline blood sample from the jugular vein was drawn and then a bolus dose of a sterile saline-urea solution (0.9% NaCl and 20% wt/vol urea; reagent grade from Fisher Chemical, Fair Lawn, NJ) was infused at 130 mg of urea/kg of BW. Another blood sample was taken 12 min after infusion from the coccygeal vein. Plasma was separated by centrifugation (2,500 × g at 4°C for 20 min) and frozen at −20°C until urea concentration was measured using a colorimetric method (UR1068, Randox Laboratories Ltd., Crumlin, UK).

Cows were also weighed when moved into box stalls, within 24 h after calving, on consecutive days when moving to tiestalls, and at 15 and 35 DIM. The BW measurements before calving were averaged, as were BW measured from 0 to 4 DIM for analysis. Body condition (1 to 5 scale) was scored during body composition measurements by 4 trained individuals and averaged for analysis.

Body Composition Calculations

Urea space volume was calculated as the quantity of urea infused divided by the difference in urea concentration from baseline and post-infusion plasma samples. Urea space volume, BCS, BW, and average milk yield from 3 d before, the day of, and 3 d after infusion were used to calculate EB CP, lipid, ash, gross energy, and water using equations 8, 9d, 11d, and 12, respectively, from Agnew et al. (2005). For prepartum urea dilution measurements, EB lipid and gross energy were estimated using equations 9c and 11c, respectively (Agnew et al., 2005) because those equations exclude milk yield as an input variable. Prepartum EB components were corrected for the conceptus (fetus, placenta, and fetal fluids) under the assumption that maternal urea equilibrates with that of the fetus (Comline and Silver, 1976). First, fetal weight at the time of urea dilution measurements was predicted using calf birth weight and back calculating to the actual day relative to calving (average ± SD primiparous: −8.1 ± 5.1 d; multiparous: −5.0 ± 3.4 d). Fetal wet weight gain was predicted as 0.42 kg/d of gestation (Bell et al., 1995). For stillborn calves, average birth weight within a parity from the experiment was used (primiparous 38 ± 4.4 kg; multiparous 44 ± 6.5 kg). Each maternal EB component was adjusted by assuming the fetus was approximately 67% of the conceptus wet weight, and that the conceptus (wet basis) was 80.8% water, 12.5% CP, 3.8% lipid, 3.0% ash, and 0.96 Mcal of gross energy/kg of BW (House and Bell, 1993). Empty body weight (**EBW**) was calculated as the sum of EB CP, lipid, water, and ash. The change in EB components was calculated as the difference in mass or energy divided by the time in days between actual measurements.

Statistical Analyses

Three cows (multigravid cow on Blend, multigravid cow on Blend-fNDF, and a heifer on Blend) calved before body composition measurements were done. Three different postpartum cows (multiparous cow on DMP at 7 DIM, multiparous cow on Blend at 25 DIM, primiparous cow on Blend-fNDF at 7 DIM) had displaced abomasums when urea dilutions were scheduled, and body composition was not measured. Another multiparous cow on DMP died unexpectedly from intestinal torsion at 49 DIM before body composition measurements were taken. Available data from these cows were used for EB composition and balance measurements. Body composition measurements that were not taken were treated as missing data.

All data were analyzed using PROC MIXED (v9.4, SAS Institute Inc., Cary, NC). For body composition data, prepartum measurements were initially tested for differences before treatment using a model with the fixed effects of postpartum treatment, parity, their interactions, and the random effect of block nested within parity. Actual day of urea dilution relative to calving and several prepartum body components differed (P < 0.05) before treatments began. Measurements were standardized using coefficients derived from a model including the effects of day relative to calving (continuous), parity, their interaction, and the random effects of block nested within parity and residual error. Measurements were then adjusted from actual day relative to calving to d −7 for each cow. After standardizing and re-analyzing prepartum data, treatment and treatment by parity interactions were no longer significant ($P \geq 0.15$; Table 2). For postpartum body component mass, data were analyzed with models including the fixed effects of treatment, parity, time relative to calving (repeated), all 2- and 3-way interactions between fixed effects, the covariate effect of standardized prepartum data and the random effect of block nested within parity and residual error. A similar model excluding the covariate effect was used to analyze change in BW, BCS, and intake, MPUE, and balance measurements. For repeated measures, covariance structures were chosen based on lowest Bayesian information criterion. For BW, BCS, and EB components, day relative to calving was the time variable, and the heterogeneous compound symmetry covariance structure was chosen. For nutrient balances, week was the time variable and the autoregressive covariance structure was used. Change in postpartum body composition was analyzed separately at each time point (i.e., prepartum to 7 DIM, 7 to 25 DIM, and 25 to 50 DIM). The model for change in postpartum body component included the fixed effects of treatment, parity, their interaction, and the random effect of block nested within parity and residual error.

The denominator degrees of freedom for all models were adjusted using the Kenward-Roger option. Orthogonal contrasts were made a priori and used to evaluate effect of MP concentration (DMP vs. AMP + Blend), AA profile (AMP vs. Blend), and fNDF (Blend vs. Blend-fNDF). The 3 contrasts plus their interaction with parity were also made. For significant treatment × time ($P < 0.10$) or treatment × time × parity ($P < 0.15$) interactions, the SLICE option was used to identify the time effect followed by a Fisher least significant difference test. Because of missing data, the highest SEM are reported for measurements.

RESULTS AND DISCUSSION

Diet concentrations of MP calculated from TMR samples assayed using in situ and mobile bag were, on average, lower than NRC (2001) library values and predictions (Table 1). Estimated (NRC 2001) RDP concentrations were similar to assayed values for DMP and AMP, but Blend and Blend-fNDF had 17% higher RDP. Because the protective coating on RP AA may be damaged, in situ disappearance of ground samples may not be accurate for RP AA; however, RP AA contributed, at most, 2.6% of the N in treatment diets. Digestibility of intestinal RUP predicted with NRC (2001) versus that assayed using the mobile bag procedure was about 6% less in DMP but similar for AMP, Blend, and Blend-fNDF. Differences in digestibility may exist between the site of bag recovery (ileum vs. fecal; Hvelplund and Weisbjerg, 2000), and our predictions of the protein truly absorbed could be biased. However, our objective was to compare the 4 treatment diets unique to this experiment. Therefore, assayed values were used for all MP calculations.

MP Balance and Utilization Efficiency During Treatment

Intake of MP increased from 1 to 4 WOL (week: $P < 0.01$; Figure 1a) for all treatments, but no treatment or parity interactions with time were found ($P \geq$

Table 2. Empty body (EB) composition during the prepartum phase (before treatments were applied) for cows fed postpartum treatments with high RUP and replacing either forage or nonforage NDF[1]

Item	Treatment[2]				SEM	P-value[3]		
	DMP	AMP	Blend	Blend-fNDF		MP	AA	fNDF
Empty BW (EBW), kg	532	527	519	515	10.9	0.50	0.60	0.76
EB water, kg	307	305	300	299	5.5	0.43	0.55	0.82
% of EBW	57.9	57.9	57.9	58.0	0.16	0.84	0.88	0.63
EB lipid, kg	110.8	110.6	109.1	107.3	3.02	0.79	0.68	0.63
% of EBW	20.8	20.9	20.9	20.7	0.17	0.50	0.81	0.50
EB CP, kg	85.9	84.8	83.7	83.2	1.73	0.39	0.63	0.81
% of EBW	16.2	16.1	16.1	16.1	0.03	0.12	0.42	0.68
EB ash, kg	27.4	27.0	26.5	26.4	0.69	0.45	0.60	0.93
% of EBW	5.12	5.10	5.09	5.12	0.03	0.46	0.76	0.47
EB gross energy, Mcal	1,596	1,589	1,567	1,542	39.1	0.69	0.67	0.63
Mcal/kg of EBW	3.00	3.01	3.01	2.99	0.02	0.42	0.93	0.44

[1]Body composition measurements calculated using the equations in Agnew et al. (2005), adjusted for the conceptus and standardized to d −7 relative to calving. A common diet was fed during the prepartum (approximately −14 to 0 DIM; 11.5% CP).

[2]Postpartum treatments were control (16.9% CP), high RUP (20.2% CP), high RUP from a blend of protein and AA sources (Blend; 19.9% CP), and Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; 19.8% CP).

[3]MP = DMP vs. AMP + Blend; AA = AMP vs. Blend; fNDF = Blend vs. Blend-fNDF.

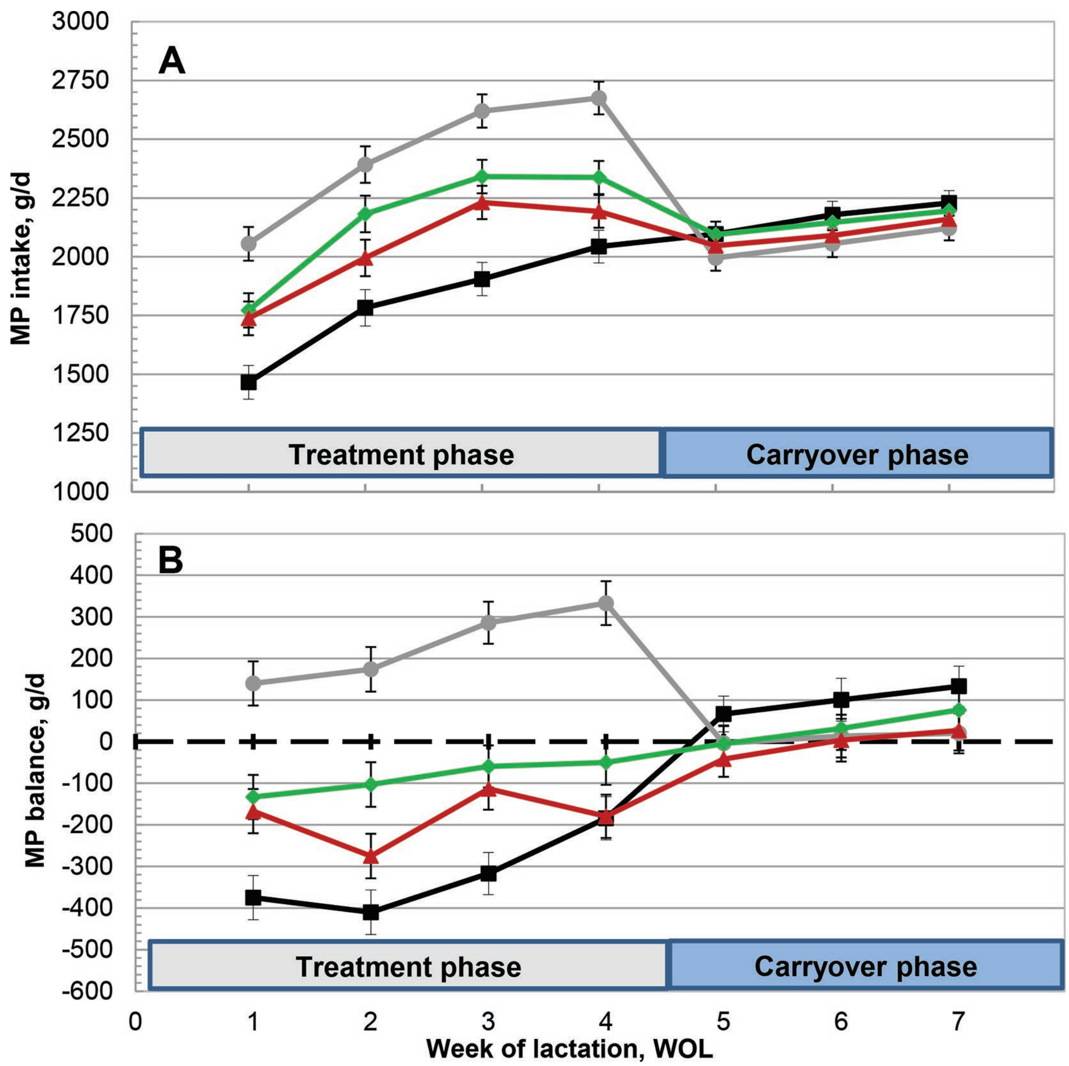

Figure 1. Effects of feeding diets with different concentrations of MP, AA profile, or forage NDF (fNDF) concentrations from 1 to 25 DIM on (A) MP intake, and (B) MP balance during the postpartum period (1 to 7 wk of lactation, WOL). A common diet was fed during the prepartum (−14 to 0 DIM) and carryover periods (26 to 50 DIM). Treatment diets (1 to 4 WOL) were deficient MP (DMP; black squares; 16.9% CP), adequate MP using primarily soy to increase RUP concentration (AMP; gray circles; 20.2% CP), adequate MP using a blend of RUP and AA sources (Blend; green diamonds; 19.9% CP), and the blend replacing forage rather than nonforage NDF sources (Blend-fNDF; red triangles; 19.8% CP). A common diet was fed during the carryover period (5 to 7 WOL; 16.3% CP). Treatment, WOL, parity, and WOL by treatment were significant ($P < 0.10$) for intake and balance. No parity by WOL or 3-way interactions were found ($P \geq 0.20$). Error bars indicate the standard error of the mean (intake = 77.0 g/d; balance = 54.6 g/d).

0.11). Estimated MP intake was lower for DMP than for AMP and Blend ($P = 0.01$; Table 3) but DMI was unaffected by MP concentration (16.4 vs. 16.8 kg/d; $P = 0.31$; Tebbe and Weiss, 2021). Altering the AA profile reduced MP intake ($P < 0.01$); however, Blend had ~1.4% (DM) lower MP concentration and greater DMI (17.3 vs. 16.2 kg/d, $P < 0.01$; Tebbe and Weiss, 2021) than AMP. No parity by MP level ($P = 0.26$) or AA profile ($P = 0.94$) interactions were found. Parity and fNDF level tended to interact for MP intake ($P = 0.07$), which followed results for DMI. For Blend-fNDF versus Blend, MP intake decreased in multiparous cows but increased in primiparous cows. On average, MP intake was about 32% lower ($P < 0.01$) for primiparous than for multiparous cows.

Predicted MP balance of treatments using the NRC (2001) MP requirements interacted with week (Figure 1b; $P = 0.001$), but no 3-way interaction was found ($P = 0.22$). For DMP, MP balance was less than zero from 1 to 4 WOL ($P < 0.01$). Compared with DMP, cows fed AMP, Blend, and Blend-fNDF had greater ($P < 0.05$) MP balances throughout the treatment phase. Cows fed AMP had positive MP balances ($P < 0.01$) throughout treatment, whereas that for Blend was less

Table 3. Effects of feeding postpartum cows diets with high RUP and replacing either forage or nonforage NDF on MP intake and balance, and MP utilization efficiency (MPUE) during treatment and carryover phases

		Treatment[2]					*P*-value[3]		
Item	Parity[1]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
Treatment phase (1 to 4 wk of lactation)									
MP intake, g/d[a]	P	1,533	2,082	1,811	1,849	87.6	0.01	0.01	0.16
	M	2,066	2,789	2,505	2,230				
MP balance, g/d[a,b]	P	−161	260	−4*	−86*	60.7	0.01	0.01	0.11
	M	−481	207	−168	−281				
Estimated MPUE,[4] %[a,b]	P	71.4	55.3	64.4	66.7	2.18	0.01	0.01	0.15
	M	82.1	59.3	69.0	73.1				
Carryover phase (5 to 7 wk of lactation)									
MP intake, g/d[a]	P	1,939	1,845	1,837	1,924	73.0	0.26	0.20	0.50
	M	2,397	2,270	2,453	2,274				
MP balance, g/d	P	207	73*	69*	85*	60.8	0.10	0.64	0.49
	M	95*	44*	101*	7*				
Estimated MPUE,[4] %	P	64.5	69.8	69.7	69.5	2.11	0.07	0.42	0.38
	M	70.9	72.8	70.0	73.4				

[a]Parity × treatment: $P < 0.10$.

[b]Week × treatment: $P < 0.10$.

[1]Parity was significant for all measures ($P < 0.05$) except MP balance in the carryover period ($P = 0.38$). P = primiparous; M = multiparous.

[2]Treatments were deficient MP (DMP; 16.9% CP), adequate MP using primarily soy to increase RUP (AMP; 20.2% CP), adequate MP using a blend of protein and AA sources to increase RUP (Blend; 19.9% CP), and Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; 19.8% CP). Treatment MP intake was calculated from the in situ incubation and the mobile bag samples and NRC (2001) MP requirements for balance measurements.

[3]MP = DMP vs. AMP + Blend; AA = AMP vs. Blend; fNDF = Blend vs. Blend-fNDF.

[4]MP utilization efficiency, % = (scurf + metabolic fecal + milk true protein secretions, g/d) ÷ (MP intake – endogenous urinary true protein secretions, g/d) × 100. Scurf, metabolic fecal, and endogenous urinary true protein requirements calculated according to Lapierre et al. (2020).

*Close to zero ($P > 0.10$).

than zero from 1 to 2 WOL ($P < 0.01$) and close to zero from 3 to 4 WOL ($P \geq 0.24$). Compared with those fed AMP, cows fed Blend and Blend-fNDF had lower MP balances from 1 to 4 WOL. Blend-fNDF had similar MP balances to Blend at 1 and 3 WOL and lower MP balances than Blend at 2 and 4 WOL ($P = 0.01$). Blend-fNDF had and MP balance less than zero ($P < 0.01$) throughout treatment. Parity interacted with MP level ($P = 0.04$) and MP balance was lower for multiparous cows on DMP versus AMP and Blend but similar among primiparous cows. The parity by MP level interaction may be because these MP balances do not include MP required for growth.

A treatment by week interaction ($P = 0.01$), but no 3-way interaction ($P = 0.53$), was found for estimated MPUE (Figure 2a and b). Utilization efficiency of MP was greater ($P = 0.01$) for DMP than for AMP and Blend. The MPUE decreased as WOL increased and MP intake increased, but the decrease was greater for DMP, causing a tendency for similar MPUE among treatments at 4 WOL ($P \geq 0.10$). Concentration of MP also tended to interact with parity on MPUE ($P = 0.09$). In multiparous cows, greater MP concentrations decreased MPUE (DMP vs. average of AMP and Blend; 82.1 vs. 64.1%) more than in primiparous cows (71.4 vs. 59.9%). Similar interactions of MP concentration with time and parity occurred for plasma concentrations of 3-methyl-His (Tebbe and Weiss, 2021). Higher MPUE for multiparous versus primiparous cows could be because (1) multiparous cows (compared with primiparous cows) mobilize more CP from endogenous stores when MP supply is deficient; (2) because primiparous cows require MP for growth (which was not included in our calculated MP requirements) plus milk (NRC, 2001); or (3) a combination of these effects. This is supported by the changes in EB CP (Figure 3).

No interactions of parity by AA profile ($P = 0.90$) or fNDF level ($P = 0.67$) were found on MPUE. Cows fed Blend versus AMP had increased MPUE (AA: $P = 0.01$). The fNDF level did not affect MPUE ($P = 0.15$). On average, MPUE across treatments was lower ($P < 0.01$) in primiparous than in multiparous cows (64.5 vs. 70.8%), which is likely because our MPUE estimates did not include MP requirements for growth.

Treatment Effects on Body Composition

During the prepartum period (Table 4), all EB components (water, lipid, CP, ash, gross energy) were greater ($P \leq 0.07$) for multiparous than for primiparous cows, but only EB ash was greater ($P = 0.01$) in multiparous cows when calculated as a proportion of EBW. The coefficients of variation (**CV**) of all EB components were numerically greater in multiparous than in primiparous cows (EB water 9.3 vs. 6.0%, lipid 14.4 vs. 8.5%, CP 10.6 vs. 6.7%, ash 13.0 vs. 7.2%, gross energy 13.0 vs. 7.6, respectively). When accounting for the range in prepartum sampling times (−19 to 0 d relative to calving) and standardizing measurements to d −7, the CV of EB components cows were reduced (EB water 8.8 vs. 5.9%, lipid 13.1 vs. 8.7%, CP 9.7 vs. 6.6%, ash 12.2 vs. 7.2%, gross energy 12.0 vs. 7.5%), whereas standardizing to EBW (e.g., % of EBW or per kg of EBW) reduced the CV considerably (EB water 1.5 vs. 0.9%, lipid 4.2 vs. 3.2%, CP 1.0 vs. 0.8%, ash 3.3 vs. 1.6%, gross energy 2.8 vs. 2.2%). The large CV in EB components among cows and highly significant effect of covariate measures ($P < 0.001$) in postpartum EB component models suggest that measurements should be taken before treatment when evaluating EB composition in dairy cows.

During treatment (0 to 25 DIM), BW change per week was negative ($P < 0.01$) but no main effects or interactions of treatment with parity and day were observed ($P \geq 0.18$; Table 5). Less fNDF tended to increase ($P = 0.08$) weekly losses of BCS, but no inter-

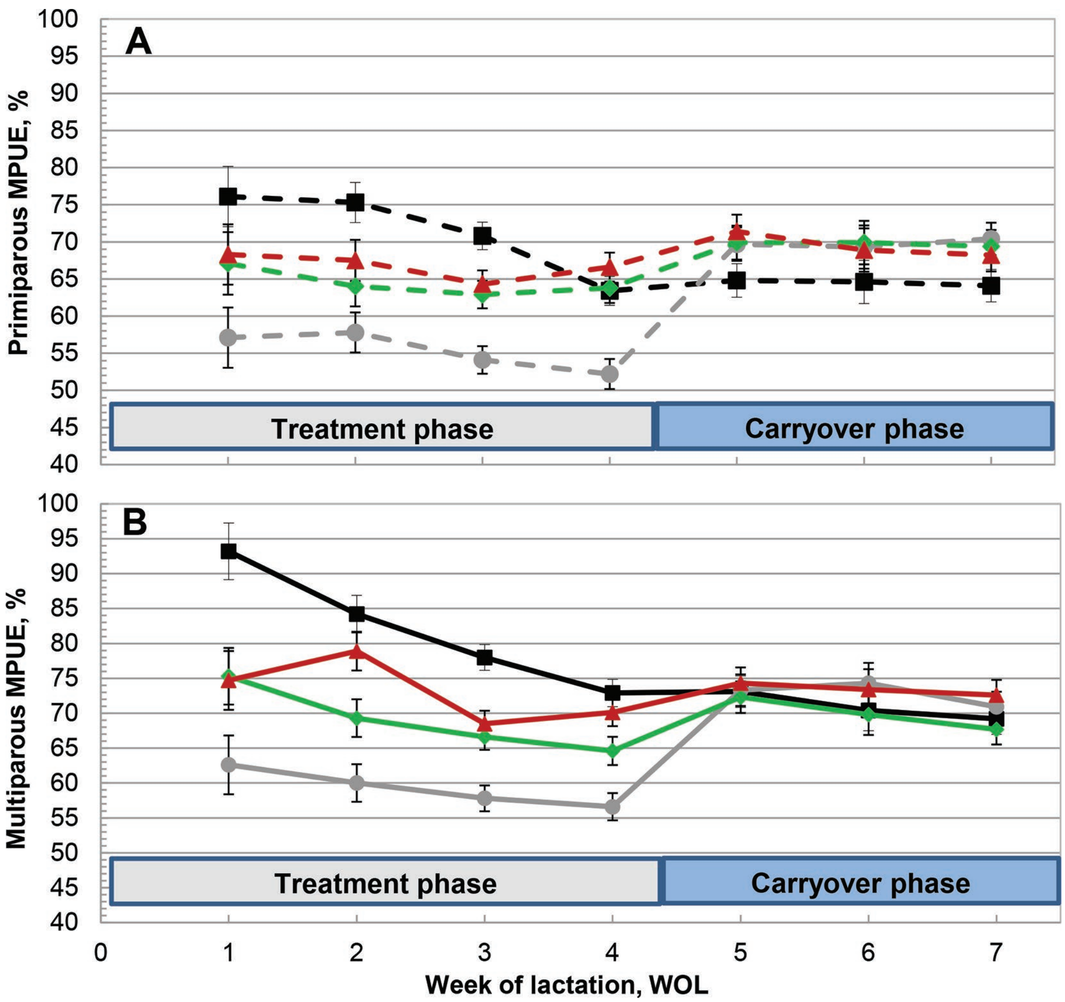

Figure 2. Concurrent and carryover effects of feeding diets with different concentrations of MP, AA profile, or forage NDF (fNDF) concentrations from 1 to 25 DIM on MP utilization efficiency [MPUE, % = (scurf + metabolic fecal + milk true protein secretions, g/d) ÷ (MP intake – endogenous urinary true protein secretions, g/d) × 100; scurf, metabolic fecal, and endogenous urinary true protein requirements calculated according to Lapierre et al. (2020)] during the postpartum period in (A) primiparous and (B) multiparous cows. Treatment diets were deficient MP (DMP; black squares; 16.9% CP), adequate MP using primarily soy to increase RUP concentration (AMP; gray circles; 20.2% CP), adequate MP using a blend of RUP and AA sources (Blend; green diamonds; 19.9% CP), and the blend replacing forage rather than nonforage NDF sources (Blend-fNDF; red triangles; 19.8% CP). A common diet was fed during the carryover period (5 to 7 WOL; 16.3% CP). Treatment, WOL, WOL by treatment, and parity were significant ($P < 0.05$) for MPUE. Parity by WOL tended to be significant ($P = 0.08$) for MPUE. No other 2- or 3-way interactions were found ($P \geq 0.10$). Error bars indicate the standard error of the mean (primiparous = 4.45; multiparous = 4.21).

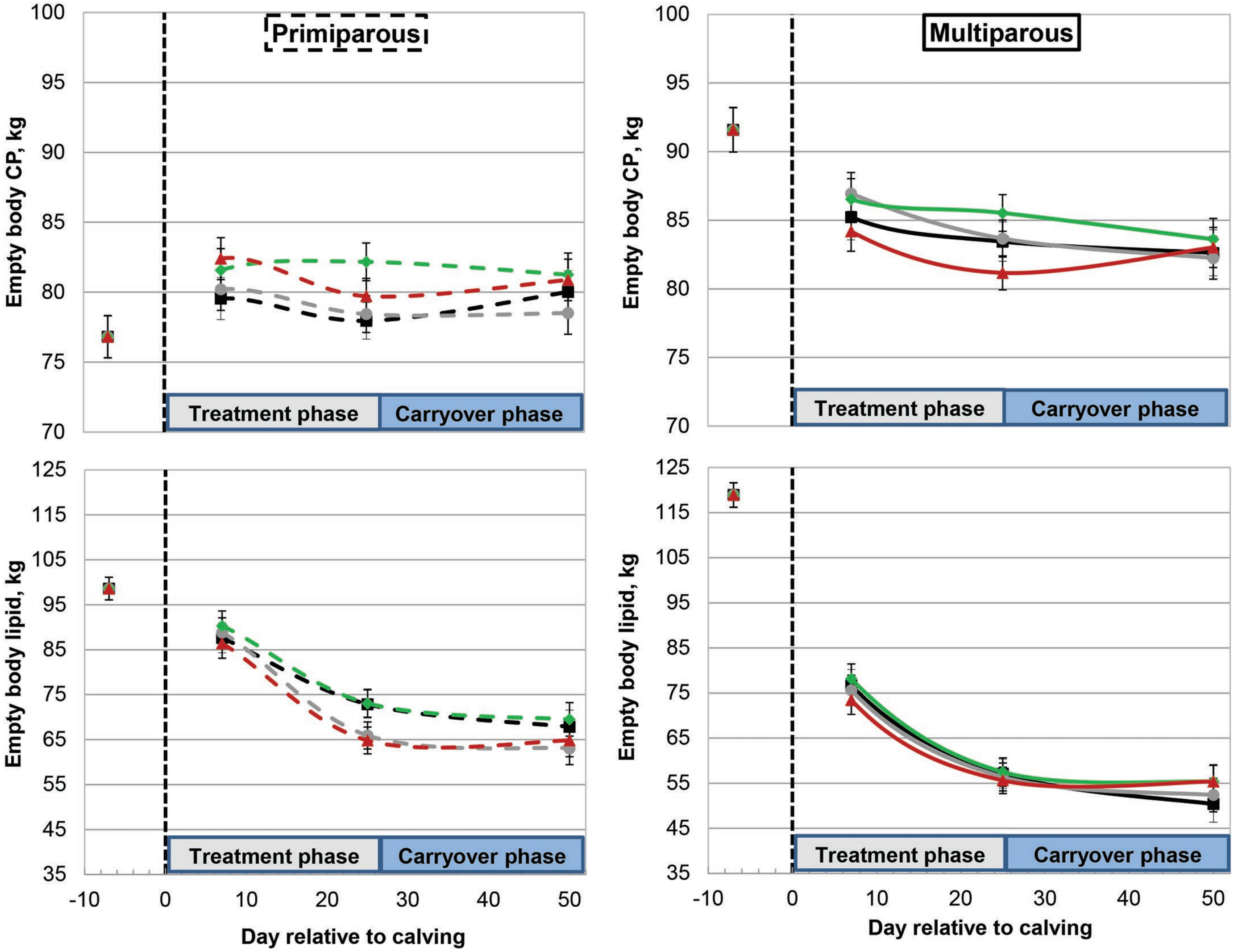

Figure 3. Effects of feeding diets with different concentrations of MP, AA profile, or forage NDF (fNDF) concentrations during the fresh period (1 to 25 DIM) on empty body CP (top row) and lipid (bottom row) in primiparous (left column, dashed lines) and multiparous cows (right column, solid lines). Treatment diets were deficient MP (DMP; black squares; 16.9% CP), adequate MP using primarily soy to increase RUP concentration (AMP; gray circles; 20.2% CP), adequate MP using a blend of RUP and AA sources (Blend; green diamonds; 19.9% CP), and the blend replacing forage rather than nonforage NDF sources (Blend-fNDF; red triangles; 19.8% CP). A common diet was fed during the prepartum (approximately −14 to 0 DIM; 11.5% CP) and carryover periods (26 to 50 DIM; 16.3% CP). Body composition was determined at −7, 7, 25, and 50 d relative to calving using the urea dilution method (Agnew et al., 2005). Day relative to calving and parity were significant ($P < 0.05$) for empty body CP and lipid. Effects of day × treatment ($P = 0.03$) and day × parity ($P = 0.04$) were found for empty body CP. No other effects or interactions were found ($P \geq 0.10$). Error bars indicate the standard error of the mean (CP = 1.68 kg; lipid = 3.72 kg).

actions of treatment by parity or main effects of MP concentration and AA profile were found. Conversely, several interactions of treatment with parity or day relative to calving were detected on EB composition (Figures 3 and 4). Measuring EB composition is likely better for evaluating nutrient mobilization than BW because of the rapid increases in DMI and gut fill and their influence on the BW of cows. Likewise, measuring EB composition rather than BCS may be better for differentiating the specific nutrients lost (i.e., lipid vs CP).

When changes in EB losses were compared with those of other published studies, our average loss of EB CP from prepartum to 50 DIM in multiparous cows (Figure 3) was lower than estimates made in peripartum cows using the urea (14 kg; Chibisa et al., 2008) and D_2O dilution methods (average 16.5 kg; Komaragiri and Erdman, 1997; Komaragiri et al., 1998) or compared with a method using lipid cell diameter and BW (8 kg; Phillips et al., 2003). Greater losses of EB CP using urea dilution or lipid cell diameter and BW could be from including conceptus BW and

Table 4. Prepartum measurements of BCS, BW, and empty body composition[1]

Item	Parity (mean ± SE)		P-value
	Primigravid	Multigravid	
BCS	3.7 ± 0.07	3.5 ± 0.06	0.04
BW, kg	660 ± 13.0	800 ± 13.3	0.02
Empty body (EB) composition[2]			
Day sampled[a]	−8.2 ± 0.64	−4.9 ± 0.65	0.01
Empty BW (EBW), kg	447 ± 9.5	534 ± 10.3	0.01
EB water, kg	278 ± 4.8	326 ± 5.1	0.01
EB lipid, kg	99 ± 2.5	119 ± 2.7	0.07
EB CP, kg	77 ± 1.5	92 ± 1.6	0.01
EB ash, kg	23.4 ± 0.56	30.1 ± 0.60	0.01
EB gross energy, Mcal	1,427 ± 32.7	1,706 ± 35.3	0.04
EB water, % of EBW	62.1 ± 0.26	61.3 ± 0.29	0.96
EB lipid, % of EBW	22.0 ± 0.14	22.2 ± 0.15	0.52
EB CP, % of EBW	17.2 ± 0.04	17.2 ± 0.05	0.19
EB ash, % of EBW	5.2 ± 0.03	5.6 ± 0.03	0.01
EB gross energy, Mcal/kg of EBW	3.19 ± 0.01	3.19 ± 0.02	0.36

[a]Parity × postpartum treatment: $P < 0.10$.

[1]Prepartum measurements taken when cows received a common close-up diet. Range in days relative to calving for measurements was −19 to −1 d relative to calving for primigravid heifers and −15 to 0 d relative to calving for multigravid cows. Measurements were analyzed with a model including the effects of d relative to calving (continuous), parity, their interaction, and the random effects of block nested within parity and residual error.

[2]Empty body composition measurements using the urea dilution method and calculated using the equations from Agnew et al. (2005). Measurements are corrected for the conceptus (see text for details). Total observations were 39 primigravid and 38 multigravid cows (multigravid cow on Blend, multigravid cow on Blend-fNDF and primigravid heifer on Blend are missing).

EB CP in prepartum EB component measurements, whereas our measurements excluded the conceptus from prepartum EB masses. The D_2O method excludes conceptus weight by deducting fetal DM weight (calf birth weight × 0.21), which was only 9.6 kg of DM in Komaragiri and Erdman (1997) and Komaragiri et al. (1998). Correction of conceptus EB CP in this study averaged 8.0 ± 1.34 kg for multiparous cows and 6.5 ± 0.82 kg for primiparous cows. Based on serial slaughter studies starting prepartum (Gibb et al., 1992; Andrew

Table 5. Effects of feeding postpartum cows diets with high RUP and replacing either forage or nonforage NDF on the average change in BCS and BW during treatment and the carryover phase[1]

Item	Parity[2]	Treatment[3]				SEM	P-value[4]		
		DMP	AMP	Blend	Blend-fNDF		MP	AA	fNDF
Treatment phase (1 to 4 wk of lactation)									
Average BW at 2 DIM, kg	P	590	587	605	608				
	M	735	728	730	691				
BW, kg/d	P	−2.63	−2.56	−2.17	−1.82	0.40	0.16	0.26	0.45
	M	−2.63	−2.89	−2.37	−3.31				
BCS, score/week	P	−0.13	−0.17	−0.13	−0.18	0.02	0.37	0.31	0.08
	M	−0.13	−0.14	−0.14	−0.17				
Carryover phase (5 to 7 wk of lactation)									
Average BW at 25 DIM, kg	P	561	564	583	584				
	M	698	698	712	655				
BW, kg/d	P	−0.20*	−0.32*	−0.47*	−0.25*	0.32	0.77	0.44	0.08
	M	−1.09	−0.89	−1.21	−0.34*				
BCS, score/week	P	−0.03*	0.00*	−0.05	−0.01*	0.02	0.23	0.42	0.13
	M	−0.08	−0.05	−0.03*	−0.01*				

[1]BCS was measured at d −7, 7, 25, and 50 DIM; BW was measured within 24 h after calving, and at 2, 3, 7, 15, 25, 35, and 50 DIM.

[2]P = primiparous; M = multiparous.

[3]Treatments were deficient MP (DMP; 16.9% CP), adequate MP using primarily soy to increase RUP (AMP; 20.2% CP), adequate MP using a blend of protein and AA sources to increase RUP (Blend; 19.9% CP), and Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; 19.8% CP).

[4]MP = DMP vs. AMP + Blend; AA = AMP vs. Blend; fNDF = Blend vs. Blend-fNDF.

*Close to zero ($P > 0.10$).

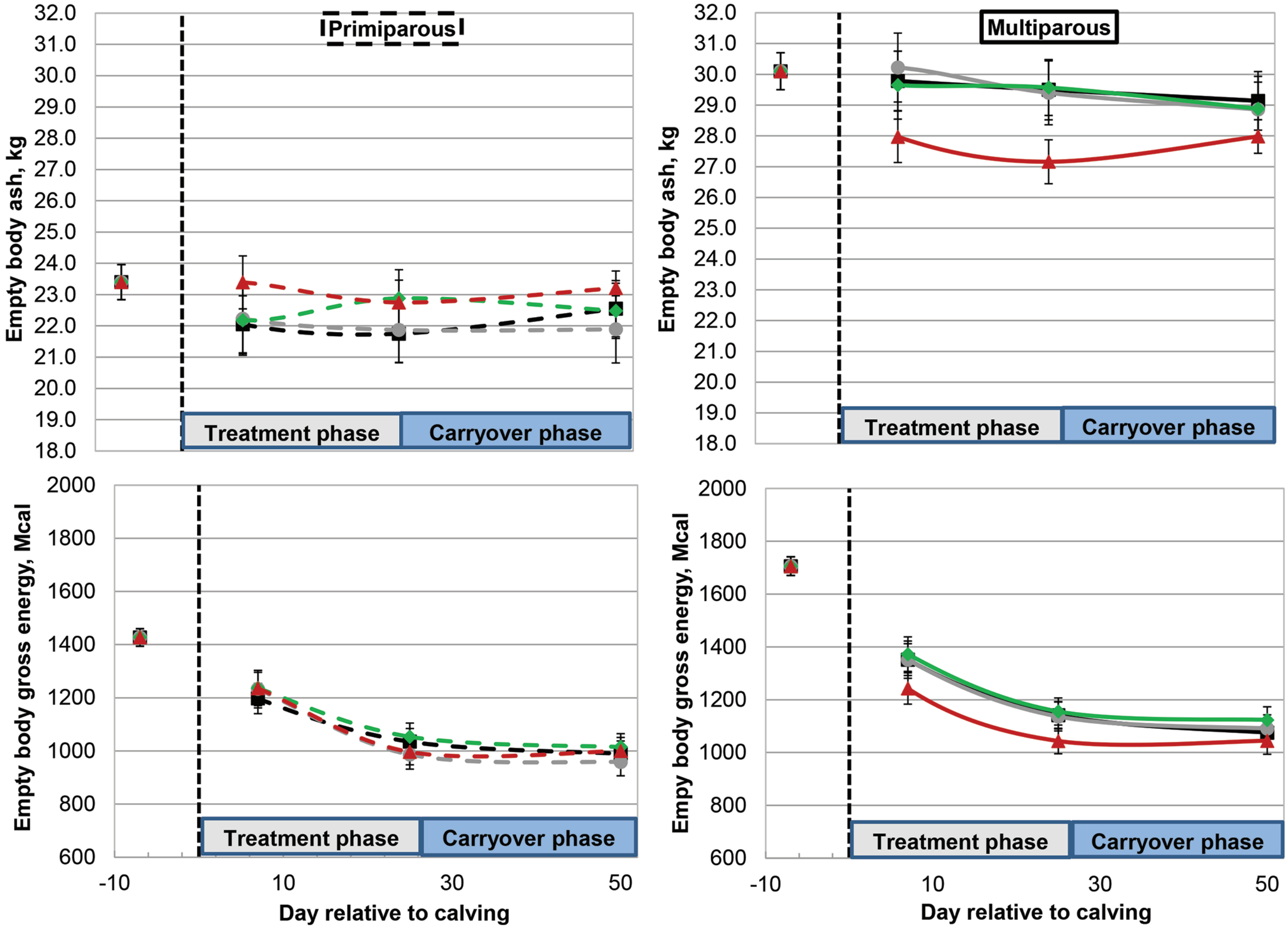

Figure 4. Effects of feeding diets with different concentrations of MP, AA profile, or forage NDF (fNDF) concentrations during the fresh period (1 to 25 DIM) on empty body ash (top row) and gross energy (bottom row) in primiparous (left column, dashed lines) and multiparous cows (right column, solid lines). Treatment diets were deficient MP (DMP; black squares; 16.9% CP), adequate MP using primarily soy to increase RUP concentration (AMP; gray circles; 20.2% CP), adequate MP using a blend of RUP and AA sources (Blend; green diamonds; 19.9% CP), and the blend replacing forage rather than nonforage NDF sources (Blend-fNDF; red triangles; 19.8% CP). A common diet was fed during the prepartum (approximately −14 to 0 DIM; 11.5% CP) and carryover periods (26 to 50 DIM; 16.3% CP). Body composition was determined at −7, 7, 25, and 50 d relative to calving using the urea dilution method (Agnew et al., 2005). Day relative to calving and parity were significant ($P < 0.05$) for empty body ash and gross energy. Effects of day × treatment ($P = 0.01$) and day × parity ($P = 0.04$) were found for empty body ash. No other effects or interactions were found ($P \geq 0.10$). Error bars indicate the standard error of the mean (ash = 1.12 kg; gross energy = 70.5 Mcal).

et al., 1994) or measuring EB components starting at 1 WOL via D_2O dilution (Chilliard et al., 1991), average EB CP losses measured in multiparous cows from prepartum or 1 WOL to about 56 DIM were similar to those in multiparous cows estimated from d −7 to 50 d relative to calving in this study (mean ± SD; 1.8 ± 3.46 vs. 2.2 ± 5.60 kg). Losses of EB lipid and gross energy were higher in serial slaughter than in multiparous cows from this study (mean ± SD; 42.2 ± 7.6 vs. 54.3 ± 13.9 kg of EB lipid; 377 ± 4.2 vs. 583 ± 163 Mcal of gross energy), but within the range reported using the other EB measurement methods (range: EB lipid 19–66 kg, EB gross energy 145–770 Mcal). Underestimating the contribution of the conceptus could overestimate the dam's starting body composition and EB losses from prepartum to postpartum. Therefore, changes in EB composition from −7 to 7 DIM were analyzed separately from 7 to 25 DIM (Table 6).

From −7 to 7 DIM, daily losses of EB water tended to be less ($P = 0.05$) with higher MP concentration. Increased MP concentrations also caused numerically lower ($P = 0.13$) daily EB CP losses. Losses of EB water and CP were not affected by MP concentration from 7 to 25 DIM. The negative MP balances from 1 to

Table 6. Effects of feeding postpartum cows diets with high RUP and replacing either forage or nonforage NDF on the change in empty body (EB) composition from −7 to 7 or from 7 to 25 d relative to calving[1]

		Treatment[3]					*P*-value[4]		
Item	Parity[2]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
−7 to 7 DIM									
EB water, kg/d	P	−1.34	−1.22	−0.91	−0.69	0.28	0.05	0.78	0.83
	M	−1.29	−0.60	−0.77	−1.10				
EB lipid, kg/d	P	−1.40	−1.42	−1.49	−1.17	0.27	0.62	0.74	0.95
	M	−2.70	−2.98	−2.75	−3.03				
EB CP, kg/d	P	−0.20	−0.11*	0.02*	−0.04*	0.10	0.13	0.92	0.79
	M	−0.04*	0.13*	−0.02*	−0.01*				
EB ash, g/d	P	−100	−80	−44*	−40*	34.9	0.29	0.79	0.85
	M	−57*	−6*	−60	−50*				
EB gross energy, Mcal/d	P	−16.1	−16.1	−16.3	−12.8	2.95	0.80	0.72	0.95
	M	−29.0	−31.2	−29.1	−32.2				
7 to 25 DIM									
EB water, kg/d	P	−0.27	−0.31	0.09*	−0.42	0.16	0.86	0.01	0.01
	M	−0.25*	−0.57	−0.16*	−0.53				
EB lipid, kg/d	P	−0.80	−1.28	−0.98	−1.14	0.13	0.07	0.32	0.89
	M	−1.04	−1.07	−1.13	−1.00				
EB CP, kg/d	P	−0.08*	−0.10	0.03*	−0.13	0.05	0.88	0.01	0.01
	M	−0.08*	−0.18	−0.05*	−0.17				
EB ash, g/d	P	−15*	−20*	39	−32*	20.0	0.84	0.01	0.01
	M	−5*	−46	−1*	−45				
EB gross energy, Mcal/d	P	−8.8	−13.7	−10.5	−12.4	1.54	0.11	0.26	0.69
	M	−11.4	−12.0	−12.1	−11.2				

[1]Empty body composition measurements using the urea dilution method and calculated using the equations in Agnew et al. (2005). Change from −7 to 7 d relative to calving is calculated as the mean between prepartum measurements corrected for the conceptus to 7 DIM.

[2]P = primiparous; M = multiparous.

[3]Treatments were deficient MP (DMP; 16.9% CP), adequate MP using primarily soy to increase RUP (AMP; 20.2% CP), adequate MP using a blend of protein and AA sources to increase RUP (Blend; 19.9% CP), and Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; 19.8% CP). Treatments were not applied until day of calving.

[4]MP = DMP vs. AMP + Blend; AA = AMP vs. Blend; fNDF = Blend vs. Blend-fNDF. No treatment by parity interactions were found ($P \geq 0.15$).

*Close to zero ($P > 0.10$).

4 WOL and higher plasma concentrations of 3-methylhistidine, a marker of skeletal muscle breakdown, found for DMP compared with AMP and Blend (Tebbe and Weiss, 2021) support greater losses of EB CP with lower MP concentrations. When feeding 19% versus 16% CP (8.4 vs. 4.5% RUP) from prepartum to 12 WOL, EB CP was similar among CP levels at 5 WOL (Komaragiri and Erdman, 1997). The lack of treatment differences for loss of EB CP in Komaragiri and Erdman (1997) could be because DMI was decreased but milk production was similar when feeding 19% versus 16% CP from prepartum to 5 WOL. The lack of effects of treatment in Komaragiri and Erdman (1997) could also be because most EB CP losses occur before 4 WOL (Martin and Ehle, 1986; Van der Drift et al., 2012).

Total EB lipid tended to have a parity by day by treatment interaction ($P = 0.14$; Figure 3). Between d −7 and 7 d relative to calving, multiparous cows lost twice as much EB lipid as primiparous cows (−2.9 vs. −1.4 kg/d; $P < 0.01$) and had less EB lipid by 7 DIM (74.9 vs. 89.3 kg; $P < 0.01$). The change in EB lipid from −7 to 7 d relative to calving was unaffected by treatments ($P \geq 0.62$). From 7 to 25 DIM, losses in EB lipid tended to be greater with greater MP concentrations ($P = 0.07$) but similar between primiparous and multiparous cows (average −1.1 kg/d; parity, $P = 0.88$). At 25 DIM, EB lipid was less in primiparous cows fed AMP and Blend-fNDF compared with Blend and DMP, whereas EB lipid was similar among treatments in multiparous cows. Similar ECM yields but greater DMI found in primiparous cows fed Blend versus AMP (Tebbe and Weiss, 2021) would explain greater EB lipid at 25 DIM. Decreased EB lipid for primiparous cows fed Blend-fNDF versus Blend occurred despite greater DMI and ECM yield (Tebbe and Weiss, 2021). Conversely, in multiparous cows, Blend-fNDF versus Blend reduced DMI and ECM yield and had no effect on EB lipid (Tebbe and Weiss, 2021).

Empty body CP had an interaction of day by AA profile ($P = 0.04$; Figure 3). At 7 DIM, EB CP was similar among treatments ($P \geq 0.20$) but greater in multiparous versus primiparous cows (85.3 vs. 81.3 kg; $P = 0.02$). From 7 to 25 DIM, losses of EB CP in both parities were greater for AMP (AA profile; $P < 0.01$)

Table 7. Effects of feeding postpartum cows diets with high RUP and replacing either forage or nonforage NDF on the change in empty body (EB) composition during the carryover period (26 to 50 DIM)[1]

Item	Parity[2]	Treatment[3]				SEM	*P*-value[4]		
		DMP	AMP	Blend	Blend-fNDF		MP	AA	fNDF
EB water, kg/d	P	0.27	0.02*	−0.11*	0.15*	0.11	0.02	0.38	0.01
	M	−0.08	−0.17	−0.23	0.23				
EB lipid, kg/d	P	−0.21	−0.11*	−0.13	−0.01*	0.07	0.07	0.98	0.06
	M	−0.29	−0.16	−0.13	−0.01*				
EB CP, kg/d	P	0.09	0.01*	−0.03*	0.05*	0.03	0.02	0.39	0.01
	M	−0.03*	−0.06*	−0.07	0.07				
EB ash, g/d	P	33	1*	−15*	19*	14.6	0.03	0.44	0.01
	M	−11	−21	−26	32				
EB gross energy, Mcal/d	P	−1.9	−1.1*	−1.5	0.1*	0.79	0.19	0.91	0.04
	M	−3.0	−1.8	−1.6	0.0*				

[1]Empty body composition measurements using the urea dilution method at 50 DIM and calculated using the equations in Agnew et al. (2005). For all measurements, change is the difference between 25 to 50 DIM.

[2]P = primiparous; M = multiparous.

[3]Treatments were deficient MP (DMP; 16.9% CP), adequate MP using primarily soy to increase RUP (AMP; 20.2% CP), adequate MP using a blend of protein and AA sources to increase RUP (Blend; 19.9% CP), and Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; 19.8% CP).

[4]MP = DMP vs. AMP + Blend; AA = AMP vs. Blend; fNDF = Blend vs. Blend-fNDF. No treatment by parity interactions were found ($P \geq 0.15$).

*Close to zero ($P > 0.10$).

and Blend-fNDF (fNDF concentration; $P < 0.01$) compared with Blend. Multiparous cows fed Blend-fNDF versus Blend had 4.3 kg less EB CP ($P = 0.01$) at 25 DIM, whereas EB CP in primiparous cows fed those 2 treatments were similar ($P = 0.12$).

Based on serial slaughter (Gibb et al., 1992; Andrew et al., 1994), total EB CP may be fairly constant from −14 to 35 d relative to calving, but the proportion of EB CP from the gastrointestinal tract, liver and udder increase as the carcass decreases. The lack of differences for plasma 3-methyl-histidine between AMP and Blend (Tebbe and Weiss, 2021) suggests that a better AA supply did not reduce mobilization of skeletal muscle. Conversely, DMI is positively associated with mass of the gastrointestinal tract and liver (Reynolds et al., 2004). Greater EB CP for Blend could result from the increased mass in the gastrointestinal tract and liver due to greater DMI. Increased proliferation of rumen papillae was found in postpartum cows infused ~500 g/d of casein from 2 to 29 DIM (Larsen et al., 2017), but DMI was similar among cows infused with casein or water (Larsen et al., 2014).

A day by treatment interaction was found for EB ash ($P = 0.06$; Figure 4). From −7 to 7 DIM, the change in EB ash was similar among treatments (average −55 g/d; $P \geq 0.29$). The change in EB ash was less from 7 to 25 DIM than from −7 to 7 DIM (−15 g/d; day: $P = 0.01$). From 7 to 25 DIM, for unknown reasons, losses of EB ash in both parities were greater for AMP and Blend-fNDF than for Blend. Multiparous cows had greater EB ash than primiparous cows (26.0 vs. 25.1 kg) throughout treatment (parity: $P = 0.05$), and losses of EB ash were similar between parities ($P = 0.83$).

No 2- or 3-way interactions ($P \geq 0.20$) and no main effects ($P \geq 0.11$) of treatment were found for losses of EB gross energy.

Carryover Effects of Treatments

When cows received a common diet (5 to 7 WOL), no carryover effects of MP concentration ($P = 0.26$) and AA profile ($P = 0.20$) or their interactions with parity ($P \geq 0.16$) were found on MP intake (Table 3). However, fNDF ($P = 0.05$) had an interaction with parity on MP intake that followed DMI. In multiparous cows, MP intake remained greater for cows previously fed Blend compared with Blend-fNDF, whereas MP intake was similar in primiparous cows. No treatment or interactions with parity were found ($P \geq 0.15$) on estimated MP balance. Intake and balance of MP increased ($P < 0.05$) as lactation progressed in the carryover period (Figure 1a and b).

Lower MP concentration during the fresh period tended to decrease ($P = 0.07$) MPUE during the carryover period. Lower MPUE for DMP (Table 3) may be a carryover effect of replenishing EB CP mobilized during treatment. This is supported by the increased ($P = 0.02$) and positive changes in EB CP during the carryover period with greater MP concentrations (Table 7). The MPUE tended to be lower in primiparous cows ($P = 0.09$) than in multiparous cows (71.8 vs. 68.4%). The 3.4-percentage-units lower MPUE is similar to the discount ($-2.5 \pm 1.20\%$) used to predict MPUE in primiparous cows (Lapierre et al., 2020) and likely results from accretion of body protein. Using average MP intake and change in EB CP of primiparous cows during the carryover period (1,886 and 25.6 g/d, respectively), 64 g/d of MP was used for growth with MPUE of 0.40 g/g. The MPUE is close to predictions of MPUE (0.38 g/g) using the NRC (2001) model for growth and using actual BW of primiparous and multiparous cows for calculations (multiparous cow average used for mature BW). Estimated MPUE decreased ($P = 0.03$) about 1 percentage unit per week from 5 to 7 WOL.

By 50 DIM, EB composition was similar across treatments ($P \geq 0.15$). Similar to losses of EB CP, losses of EB water and ash were decreased ($P \leq 0.03$) for cows fed a greater MP concentration during treatment. Conversely, losses of EB lipid tended to be greater ($P = 0.07$) with greater MP concentrations. The AA profile fed during treatment did not affect change in body composition during the carryover period. Change in EB water, CP, ash, and gross energy were greater ($P \leq 0.04$) and lipid tended to be greater ($P = 0.06$) during the carryover period for cows fed less fNDF during treatment.

No parity by treatment ($P \geq 0.14$) or main effects of treatment ($P \geq 0.13$) were found for change in BCS during the carryover period (Table 5). Change in BW during the carryover period tended to be greater ($P = 0.08$) for cows fed less fNDF during treatment. No parity by treatment ($P \geq 0.29$) or main effects of MP concentration ($P = 0.77$) and AA profile ($P = 0.44$) were found for BW change during the carryover period.

Because of the variation in EB composition among cows, postpartum EB composition as a proportion of EBW was calculated (Table 8). No treatment effects or interactions with parity and day were found ($P \geq 0.15$) for any EB components as a proportion of EBW. Proportions of EB water, CP, lipid, and energy to EBW had day by parity interactions ($P \leq 0.03$; data not shown). From 7 to 25 DIM, EB lipid as a % of EBW and EB gross energy/kg of EBW were decreased, whereas EB water and CP as a % of EBW increased in both parities. From 25 to 50 DIM, EB lipid % of EBW and EB gross energy/kg of EBW continued to decrease, whereas EB water and CP % of EBW continued to increase in primiparous cows; all EB proportions in multiparous cows were similar at 25 and 50 DIM. Multiparous cows also had decreased (parity: $P \leq 0.01$)

Table 8. Effects of feeding postpartum cows diets with high RUP and replacing either forage or nonforage NDF on empty body (EB) weight and empty body composition (% of EB weight) at 7, 25, or 50 DIM[1]

Item	Day	Treatment[2] DMP	AMP	Blend	Blend-fNDF	SEM	P-value[3] MP	AA	fNDF
EB weight (EBW),[a] kg	7[x]	474	479	480	474	6.7	0.63	0.22	0.24
	25[xy]	450[bc]	447[c]	460[b]	443[c]				
	50[y]	447	441	452	451				
EB water, % of EBW	7[x]	59.9	60.1	60.1	60.5	0.31	0.29	0.52	0.28
	25[y]	62.0	62.7	62.4	62.9				
	50[z]	62.8	63.1	62.8	62.9				
EB lipid, % of EBW	7[x]	17.4	17.1	17.2	16.6	0.42	0.25	0.46	0.28
	25[y]	14.6	13.6	13.9	13.4				
	50[z]	13.3	13.0	13.5	13.2				
EB CP, % of EBW	7[x]	17.3	17.4	17.3	17.5	0.08	0.47	0.45	0.20
	25[y]	17.9	18.1	18.0	18.1				
	50[z]	18.2	18.2	18.1	18.2				
EB ash, % of EBW	7[x]	5.36	5.39	5.35	5.41	0.03	0.61	0.52	0.40
	25[y]	5.59	5.65	5.65	5.64				
	50[z]	5.68	5.67	5.65	5.69				
EB gross energy, Mcal/kg of EBW	7[x]	2.69	2.66	2.68	2.62	0.04	0.32	0.44	0.22
	25[y]	2.42	2.33	2.36	2.31				
	50[z]	2.31	2.28	2.33	2.29				

[a]Day × treatment: $P < 0.10$.

[b,c]Values in the same row followed by different superscripts are significantly different ($P < 0.05$).

[x–z]Average values for a day in the same column and EB component followed by different superscripts differ ($P < 0.05$).

[1]Empty body composition measurements using the urea dilution method at 7, 25, and 50 DIM and calculated using the equations in Agnew et al. (2005). Empty BW, kg = EB water, kg + EB lipid, kg + EB CP, kg + EB ash, kg. No parity interactions with treatment were found ($P > 0.10$).

[2]Treatments were control (16.9% CP), high RUP (20.2% CP), high RUP from a blend of protein and AA sources (Blend; 19.9% CP), and Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; 19.8% CP).

[3]MP = DMP vs. AMP + Blend; AA = AMP vs. Blend; fNDF = Blend vs. Blend-fNDF. No treatment by time interactions were found ($P \geq 0.15$).

EB lipid % of EBW and EB gross energy/kg of EBW and increased (parity: $P \leq 0.01$) EB water and CP % of EBW at 7, 25, and 50 DIM compared with primiparous cows.

CONCLUSIONS

Feeding fresh cows a high MP diet with a balanced AA profile increased DMI and reduced EB losses of CP. However, partitioning greater energy from the greater DMI differed among parities, with primiparous cows utilizing greater supply to spare lipid mobilization and multiparous cows utilizing greater supply for milk production. Increasing MP at the expense of forage NDF caused more tissue mobilization, with primiparous cows having reduced EB lipid and multiparous cows having reduced EB CP. Greater tissue mobilization with less fNDF led to more tissue accretion during the carryover period. Reduced EB CP but similar losses of EB lipid over time suggested that greater MP concentrations, AA profile, and fNDF concentration had no influence on lipid mobilization in multiparous cows. Overall, results indicate that parity, AA supply, and fNDF concentrations fed to fresh cows can influence concurrent and longer-term partitioning of nutrients to tissues and milk production.

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REFERENCES

Agnew, R. E., T. Yan, W. McCaughey, J. McEvoy, D. Patterson, M. Porter, and R. Steen. 2005. Relationships between urea dilution measurements and body weight and composition of lactating dairy cows. J. Dairy Sci. 88:2476–2486. https://doi.org/10.3168/jds.S0022-0302(05)72925-8.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. https://doi.org/10.3168/jds.S0022-0302(00)75030-2.

Amanlou, H., T. A. Farahani, and N. E. Farsuni. 2017. Effects of rumen undegradable protein supplementation on productive performance and indicators of protein and energy metabolism in Holstein fresh cows. J. Dairy Sci. 100:3628–3640. https://doi.org/10.3168/jds.2016-11794.

Andrew, S. M., D. Waldo, and R. Erdman. 1994. Direct analysis of body composition of dairy cows at three physiological stages. J. Dairy Sci. 77:3022–3033. https://doi.org/10.3168/jds.S0022-0302(94)77244-1.

Bell, A. W., R. Slepetis, and U. Ehrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy Sci. 78:1954–1961. https://doi.org/10.3168/jds.S0022-0302(95)76821-7.

Bell, A. W., W. S. Burhans, and T. R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. Proc. Nutr. Soc. 59:119–126. https://doi.org/10.1017/S0029665100000148.

Carder, E. G., and W. Weiss. 2017. Short-and longer-term effects of feeding increased metabolizable protein with or without an altered amino acid profile to dairy cows immediately postpartum. J. Dairy Sci. 100:4528–4538. https://doi.org/10.3168/jds.2016-12362.

Chibisa, G. E., G. Gozho, A. Van Kessel, A. Olkowski, and T. Mutsvangwa. 2008. Effects of peripartum propylene glycol supplementation on nitrogen metabolism, body composition, and gene expression for the major protein degradation pathways in skeletal muscle in dairy cows. J. Dairy Sci. 91:3512–3527. https://doi.org/10.3168/jds.2007-0920.

Chilliard, Y., M. Cisse, R. Lefaivre, and B. Remond. 1991. Body composition of dairy cows according to lactation stage, somatotropin treatment, and concentrate supplementation. J. Dairy Sci. 74:3103–3116. https://doi.org/10.3168/jds.S0022-0302(91)78496-8.

Comline, R. S., and M. Silver. 1976. Some aspects of foetal and utero-placental metabolism in cows with indwelling umbilical and uterine vascular catheters. J. Physiol. 260:571–586. https://doi.org/10.1113/jphysiol.1976.sp011532.

Giallongo, F., M. Harper, J. Oh, J. Lopes, H. Lapierre, R. Patton, C. Parys, I. Shinzato, and A. Hristov. 2016. Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows. J. Dairy Sci. 99:4437–4452. https://doi.org/10.3168/jds.2015-10822.

Gibb, M., W. Ivings, M. Dhanoa, and J. Sutton. 1992. Changes in body components of autumn-calving Holstein-Friesian cows over the first 29 weeks of lactation. Anim. Prod. 55:339–360. https://doi.org/10.1017/S0003356100021036.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci. 73:2820–2833. https://doi.org/10.2527/1995.7392820x.

House, W. A., and A. W. Bell. 1993. Mineral accretion in the fetus and adnexa during late gestation in Holstein cows. J. Dairy Sci. 76:2999–3010. https://doi.org/10.3168/jds.S0022-0302(93)77639-0.

Hvelplund, T., and M. R. Weisbjerg. 2000. In situ techniques for the estimation of protein degradability and postrumen availability. Pages 233–258 in Forage Evaluation in Ruminant Nutrition. D. I. Given, E. Owen, R. F. E. Axford, and H. M. Omed, ed. CABI Publishing, New York, NY.

Komaragiri, M. V., D. Casper, and R. Erdman. 1998. Factors affecting body tissue mobilization in early lactation dairy cows. 2. Effect of dietary fat on mobilization of body fat and protein. J. Dairy Sci. 81:169–175. https://doi.org/10.3168/jds.S0022-0302(98)75564-X.

Komaragiri, M. V., and R. A. Erdman. 1997. Factors affecting body tissue mobilization in early lactation dairy cows. 1. Effect of dietary protein on mobilization of body fat and protein. J. Dairy Sci. 80:929–937. https://doi.org/10.3168/jds.S0022-0302(97)76016-8.

Kononoff, P. J., S. Ivan, and T. J. Klopfenstein. 2007. Estimation of the proportion of feed protein digested in the small intestine of cattle consuming wet corn gluten feed. J. Dairy Sci. 90:2377–2385. https://doi.org/10.3168/jds.2006-552.

Lapierre, H., R. Martineau, M. Hanigan, H. Van Lingen, E. Kebreab, J. Spek, and D. Ouellet. 2020. Impact of protein and energy supply on the fate of amino acids from absorption to milk protein in dairy cows. Animal 14:s87–s102. https://doi.org/10.1017/S1751731119003173.

Larsen, M., H. Lapierre, and N. B. Kristensen. 2014. Abomasal protein infusion in postpartum transition dairy cows: Effect on performance and mammary metabolism. J. Dairy Sci. 97:5608–5622. https://doi.org/10.3168/jds.2013-7247.

Larsen, M., C. M. Røntved, P. K. Theil, M. Khatun, C. Lauridsen, and N. B. Kristensen. 2017. Effect of experimentally increased protein supply to postpartum dairy cows on plasma protein synthesis, rumen tissue proliferation, and immune homeostasis. J. Anim. Sci. 95:2097–2110. https://doi.org/10.2527/jas2016.1055.

Martin, R. A., and F. Ehle. 1986. Body composition of lactating and dry Holstein cows estimated by deuterium dilution. J. Dairy Sci. 69:88–98. https://doi.org/10.3168/jds.S0022-0302(86)80373-3.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Paz, H. A., T. Klopfenstein, D. Hostetler, S. C. Fernando, E. Castillo-Lopez, and P. Kononoff. 2014. Ruminal degradation and intestinal digestibility of protein and amino acids in high-protein feedstuffs commonly used in dairy diets. J. Dairy Sci. 97:6485–6498. https://doi.org/10.3168/jds.2014-8108.

Phillips, G. J., T. Citron, J. Sage, K. Cummins, M. Cecava, and J. McNamara. 2003. Adaptations in body muscle and fat in transition dairy cattle fed differing amounts of protein and methionine hydroxy analog. J. Dairy Sci. 86:3634–3647. https://doi.org/10.3168/jds.S0022-0302(03)73969-1.

Piantoni, P., A. Lock, and M. Allen. 2015. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum and carryover periods in Holstein cows: Production responses and digestibility of nutrients. J. Dairy Sci. 98:3309–3322. https://doi.org/10.3168/jds.2014-8798.

Reynolds, C. K., B. Dürst, B. Lupoli, D. Humphries, and D. Beever. 2004. Visceral tissue mass and rumen volume in dairy cows during the transition from late gestation to early lactation. J. Dairy Sci. 87:961–971. https://doi.org/10.3168/jds.S0022-0302(04)73240-3.

Schei, I., H. Volden, and L. Bævre. 2005. Effects of energy balance and metabolizable protein level on tissue mobilization and milk performance of dairy cows in early lactation. Livest. Prod. Sci. 95:35–47. https://doi.org/10.1016/j.livprodsci.2004.11.039.

Tebbe, A. W., and W. P. Weiss. 2020. Effects of oscillating dietary crude protein concentrations on production, nutrient digestion, plasma metabolites, and body composition in lactating dairy cows. J. Dairy Sci. 103:10219–10232. https://doi.org/10.3168/jds.2020-18613.

Tebbe, A. W., and W. P. Weiss. 2021. Concurrent and carryover effects of feeding blends of protein and amino acids in high-protein diets with different concentrations of forage fiber to fresh cows. 1. Production and blood metabolites. J. Dairy Sci. 104:5583–5600. https://doi.org/10.3168/jds.2020-19663.

van der Drift, S. G., M. Houweling, J. Schonewille, A. Tielens, and R. Jorritsma. 2012. Protein and fat mobilization and associations with serum β-hydroxybutyrate concentrations in dairy cows. J. Dairy Sci. 95:4911–4920. https://doi.org/10.3168/jds.2011-4771.

ORCIDS

A. W. Tebbe https://orcid.org/0000-0002-7366-4065
W. P. Weiss https://orcid.org/0000-0003-3506-4672