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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. 1. Production and blood metabolites

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ABSTRACT

Because of low feed intake during the first weeks of lactation, dietary concentration of metabolizable protein (MP) must be elevated. We evaluated effects of providing additional rumen-undegradable protein (RUP) from a single source or a blend of protein and AA sources during the first 3 wk of lactation. We also evaluated whether replacing forage fiber (fNDF) or nonforage fiber with the blend affected responses. In a randomized block design, at approximately 2 wk prepartum, 40 primigravid (664 ± 44 kg of body weight) and 40 multigravid (797 ± 81 kg of body weight) Holsteins were blocked by calving date and fed a common diet (11.5% crude protein, CP). After calving to 25 d in milk (DIM), cows were fed 1 of 4 diets formulated to be (1) 20% deficient in metabolizable protein (MP) based on predicted milk production (17% CP, 24% fNDF), (2) adequate in MP using primarily RUP from soy to increase MP concentration (AMP; 20% CP, 24% fNDF), (3) adequate in MP using a blend of RUP and rumen-protected AA sources to increase MP concentration (Blend; 20% CP, 24% fNDF), or (4) similar to Blend but substituting fNDF with added RUP rather than nonforage neutral detergent fiber (Blend-fNDF; 20% CP, 19% fNDF). The blend was formulated to have a RUP supply with an AA profile similar to that of casein. A common diet (17% CP) was fed from 26 to 92 DIM, and milk production and composition were measured from 26 to 92 DIM, but individual dry matter intake (DMI) was measured only until 50 DIM. During the treatment period for both parities, AMP and Blend increased energy-corrected milk (ECM) yields compared with the diet deficient in MP based on predicted milk production (40.7 vs. 37.8 kg/d) and reduced concentrations of plasma 3-methyl-His (4.1 vs. 5.3 μmol/L) and growth hormone (9.0 vs. 11.9 ng/mL). Blend had greater DMI than AMP (17.4 vs. 16.1 kg/d), but ECM yields were similar. Blend had greater plasma Met (42.0 vs. 26.4 μmol/L) and altered metabolites associated with antioxidant production and methyl donation compared with AMP. Conversely, the concentration of total essential AA in plasma was less in Blend versus AMP (837 vs. 935 μmol/L). In multiparous cows, Blend-fNDF decreased DMI and ECM yield compared with Blend (19.2 vs. 20.1 kg/d of DMI, 45.3 vs. 51.1 kg/d of ECM), whereas primiparous cows showed the opposite response (15.3 vs. 14.6 kg/d of DMI, 32.9 vs. 31.4 kg/d of ECM). Greater DMI for multiparous cows fed Blend carried over from 26 to 50 DIM and was greater compared with AMP (23.1 vs. 21.2 kg /d) and Blend-fNDF (21.3 kg/d). Blend also increased ECM yield compared with AMP (49.2 vs. 43.5 kg/d) and Blend-fNDF (45.4 kg/d) from 26 to 92 DIM. Few carryover effects of fresh cow treatments on production were found in primiparous cows. Overall, feeding blends of RUP and AA may improve the balance of AA for fresh cows fed high MP diets and improve concurrent and longer-term milk production in multiparous cows. However, with high MP diets, multiparous fresh cows require greater concentrations of fNDF than primiparous cows.

Key words: rumen-undegradable protein, plasma amino acids, forage neutral detergent fiber, casein, parity

INTRODUCTION

Increasing MP and AA supply may be of greater importance for fresh cows than dietary energy supply because postpartum cows have a greater capacity to mobilize body lipid versus protein (Schei et al., 2005). Positive production responses to increased MP supply or a balanced AA profile may also carry over later into lactation, after treatments end (Carder and Weiss, 2017).

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Cows fed 10.1 to 13.7% MP of DM via increasing RUP concentration (5.0 to 9.0% RUP) from a variety of protein sources increased FCM yields 15% and DMI 8.5% during the first 3 wk of lactation (Amanlou et al., 2017). However, increasing RUP using a single source, which could occur with least-cost diet formulation, can exacerbate AA imbalances because RUP dilutes a more balanced AA supply coming from microbial protein (Schingoethe, 1996; Santos et al., 1998). Using blends of protein and rumen-protected (**RP**) AA sources to optimize the RUP supply to a more ideal AA profile, such as casein (Larsen et al., 2015), may improve the AA nutrition of fresh cows. Adding the blend of protein high in RUP and reducing forage NDF (**fNDF**) instead of nonforage NDF could improve DMI (Allen, 2000) and increase supply of all nutrients. Effects of increased AA supply or DMI on milk production may differ between parities because primiparous cows have MP requirements for growth in addition to lactation (NRC, 2001), and DMI as a percent of BW is lower in primiparous versus multiparous cows (Reshalaitihan et al., 2020).

The objectives of this experiment were to evaluate concurrent and carryover production responses in postpartum cows when dietary MP was increased using mostly soy protein or blends of protein and AA sources and by replacing fNDF or nonforage NDF. We hypothesized a greater MP supply from a blend of protein and AA sources would improve concurrent and carryover yields of milk and milk components compared with soy, and the increase in DMI and production would be greater when replacing the blend at the expense of forage rather than nonforage NDF sources. We further hypothesized that treatments and parity will interact on production and increase component yields more for multiparous cows because primiparous cows require MP for growth plus lactation and have lower DMI as a percent of BW after calving.

MATERIALS AND METHODS

Cows, Animal Care, and Treatments

All procedures involving animals were approved by The Ohio State University Institutional Animal Care and Use Committee (Protocol #2018A00000093). Forty primigravid heifers 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 diet (55.8% NDF, 11.5% CP, 1.37 Mcal/kg NE_L; Tebbe, 2020). Immediately after calving, cows began treatment and were fed 1 of 4 diets (Table 1) until 25 DIM in tiestalls. At 26 DIM, cows remained in their tiestalls, began the carryover phase, and were fed a common diet formulated to meet 105% of NRC (2001) predicted MP requirements. At 51 DIM, primiparous and multiparous cows were moved to separate freestall pens but fed the same carryover diet. The tiestall and freestall carryover diets were similar (Tebbe, 2020). Collection of milk and milk component yield data ended at 92 DIM.

In box- and tiestalls, diets were fed once daily (0700 h) at a refusal rate of 5 to 7%. In freestall pens, diets were fed once daily (1100 h) at a group refusal rate of 5%. Cow density in pens (30 stalls per pen) was maintained at 65 to 100% of capacity using nonexperimental cows. Silages were sampled weekly and analyzed for DM (100°C for 48 h) to adjust diets for changes in silage DM.

Treatment diets were formulated (NRC, 2001) for fresh cows with estimated DMI of 16 kg and milk yield of 34 kg at 3.9% fat and 3.2% protein (Tables 1 and 2). Diets were balanced for similar RDP concentrations and to exceed mineral and vitamin requirements. Because of consistent health and production benefits of supplementing RP-Met to peripartum cows (McFadden et al., 2020), all treatment diets were also formulated to provide at least 9.6 g/d of supplemental metabolizable Met. Treatments were (1) a diet formulated to be 20% deficient in MP (**DMP**), (2) a diet designed to be adequate in MP using primarily lignosulfonate-treated soybean meal to increase RUP (**AMP**), (3) a diet designed to meet MP requirements using a blend of feed ingredients high in RUP and RP AA (Blend), and (4) a diet similar to Blend but substituting RUP and RP AA for forages rather than nonforage fiber sources (Blend-fNDF). Diets with adequate MP were balanced for similar CP, RUP, NDF, and starch but differed in either AA composition or fNDF (Table 2).

The AA profile in the RUP of the Blend treatment was designed to resemble the EAA profile of casein using the Solver function in Excel (Microsoft Corp., Seattle, WA). Plant-based protein sources high in RUP and low in fat were selected and their AA profiles entered along with RP-Met, RP-Lys, and RP-His. Then, the Solver function was used to solve the least sum of squares difference between the EAA profile of casein and the AA profile of a solution, with the constraint that the solution's composition equal 100%. To determine the ingredient composition of the Blend, the solution's composition was put on a RUP basis using rumen degradabilities of N provided by the manufacturers or NRC (2001; corn gluten meal only).

Table 1. Ingredient composition of diets (% of DM) fed during treatment and carryover phases[1]

	Treatment[2]				
Ingredient	DMP	AMP	Blend	Blend-fNDF	Carryover
Corn silage	40.0	39.8	40.1	30.7	39.8
Alfalfa silage	12.3	12.6	12.1	9.6	11.1
Alfalfa hay	6.8	6.8	6.8	6.6	6.8
Whole cottonseed	0.0	0.0	0.0	0.0	9.2
Corn grain, ground	12.2	10.4	10.3	15.4	15.2
Soybean meal (SBM), 48% CP	17.7	15.0	12.7	12.8	13.1
Lignosulfonate-treated SBM[3]	0.0	11.4	0.0	0.0	1.31
Protein and AA blend[4]	0.0	0.0	13.9	13.8	0.0
Soy hulls	4.01	0.0	0.0	4.02	0.1
Beet pulp, dried	2.99	0.0	0.0	2.99	0.0
Animal/vegetable fat	0.42	0.42	0.42	0.42	0.41
Rumen-protected Met[5]	0.10	0.10	0.10	0.10	0.0
Mineral/vitamins treatment[6]	3.55	3.55	3.55	3.55	0.0
Mineral/vitamins carryover[7]	0.0	0.0	0.0	0.0	3.04

[1]Cows were fed 1 of 4 treatment diets immediately after calving until 25 DIM. During the carryover phase (25–92 DIM), all cows were fed the carryover diet from 25 to 50 DIM in tiestalls. Cows were fed a similar diet as the carryover from 51 to 92 DIM in group freestall pens (Tebbe, 2020).

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

[3]Surepro (Land O'Lakes Purina Feed LLC, St. Paul, MN).

[4]Contained 76.9% AminoMax Pro (Afgritech LLC, Watertown, NY), 18.0% corn gluten meal, 4.0% RP-Lys (Aminoshure-L, Balchem Corp., New Hampton, NY), 0.57% RP-Met (Smartamine M, Adisseo Inc., Antony, France), and 0.52% RP-His (experimental RP L-His·HCl product; Balchem Corp.).

[5]Smartamine M (Adisseo Inc.)

[6]Premix contained 28.7% limestone, 16.9% trace mineral salt (Morton Salt Inc., Chicago, IL), 16.7% Kcarb+ (Origination O_2D, Sioux City, IA), 6.8% magnesium oxide (Animag Prilled 30/100, Martin Marietta Magnesia Specialties LLC, Baltimore, MD), 5.1% sodium selenate premix (200 mg of Se/kg), 6.8% monosodium phosphate, 0.08% copper sulfate, 0.52% Zinpro 120 (120 g of Zn/kg, Zinpro Corp., Eden Prairie, MN), 0.46% vitamin A (30 kIU/g), 1.7% vitamin D (3 kIU/g), 5.1% vitamin E (44 kIU/kg), 11.0% biotin premix (220 mg/kg), and 0.25% Rumensin-90 (Elanco Animal Health, Greenfield, IN).

[7]Premix contained 32.2% limestone, 19.3% trace mineral salt, 12.7% Kcarb+, 8.1% magnesium oxide, 5.8% sodium selenate premix (200 mg of Se/kg), 6.3% monosodium phosphate, 0.09% copper sulfate, 0.25% Zinpro 120, 0.34% vitamin A (30 kIU/g), 1.1% vitamin D (3 kIU/g), 1.5% vitamin E (44 kIU/kg), 12.1% biotin premix (220 mg/kg), and 0.25% Rumensin-90.

Measurements, Sampling, and Laboratory Analyses

Feed delivery and refusal amounts were weighed and recorded daily for each cow. Refusal samples were taken during wk 1, 3, and 6 after calving, analyzed for DM (100°C for 48 h), and used to calculate DMI in tiestalls. Feed ingredients were sampled weekly, composited monthly and assayed for DM (100°C for 48 h). Silage composite samples were dried (55°C for 48 h) and ground through a 1-mm screen (Wiley mill; Arthur H. Thomas Co., Philadelphia, PA). Dry hay and subsamples of concentrate mixes were ground through a 1-mm screen. Ground samples of forages and unground concentrate mixes were assayed for DM (100°C for 24 h), ash (muffle oven at 600°C overnight), CP (Kjeldahl N × 6.25; method 984.13.4.09; AOAC International, 2000), long-chain fatty acids (Weiss and Wyatt, 2003), 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 then ashed (neutral detergent insoluble ash, **NDI-ash**; muffle oven at 600°C overnight) or analyzed for CP (neutral detergent insoluble CP, **NDICP**; Kjeldahl N × 6.25) to calculate NDF as ash- and CP-free ($\mathbf{NDF_{om+cp}}$). Dried, ground feed samples were composited bimonthly and analyzed for minerals, by the Ohio Agricultural Research and Development Center (OARDC) STAR Laboratory (Wooster, OH), and starch (Weiss and Wyatt, 2000) with modifications (Tebbe et al., 2018).

Cows were milked twice daily at 0400 and 1600 h. Milk yields were measured using electronic milk meters (Afimilk; Kibbutz Afikim, Israel) in tie- and freestalls. Composite milk samples (a.m. and p.m.) were collected once weekly and analyzed for milk fat, true protein, and lactose (B2000 Infrared Analyzer, Bentley Instruments, Chaska MN), and MUN (Skalar SAN Plus segmented flow analyzer, Skalar Inc., Norcross, GA) by DHI Cooperative Inc. (Columbus, OH). Additional a.m. milk samples were collected at 7, 25, and 50 DIM for fatty

acid (**FA**) analyses. The milk fat layer was removed after centrifugation (17,000 × *g* at 4°C for 30 min), and milk FA profile determined using a 2-step procedure for methylation (Jenkins, 2000) with separation by GLC using a CP-SIL88 capillary column (100 m × 0.25 mm × 0.2 μm film thickness; Varian Inc., Palo Alto, CA).

Cows were weighed and body condition was scored regularly during the experiment; those data along with body composition are reported in the companion paper (Tebbe and Weiss, 2021). Average BW at 1 DIM were 721 and 598 kg for multiparous and primiparous cows (not affected by treatment; Tebbe and Weiss, 2021).

Blood (~10 mL) was collected shortly before feeding from the tail vessels at 4, 7, 10, 25, and 50 DIM. Blood was distributed equally into sodium heparin and K_2EDTA Vacutainers (BD Vacutainer, Franklin Lakes, NJ). Plasma was separated by centrifugation (2,500 × *g* at 4°C for 20 min) and frozen at −20°C (heparinized plasma) or −80°C (K_2EDTA plasma) before analyses. Analyses using heparinized plasma included albumin (Albumin Liquicolor No. 0285), glucose (Glucose Liquicolor No. 1070), BHB (β-hydroxybutyrate Liquicolor No. 2440; Stanbio Laboratory, Boerne, TX), creatinine (Creatinine Colorimetric Assay no. 700460; Cayman Chemical Co., Ann Arbor, MI), and nonesterified FA [NEFA-HR(2); Wako Chemicals, Richmond, VA]. Subsamples of plasma from 16 random blocks (8 primiparous and 8 multiparous blocks) at 7 and 25 DIM were analyzed for AA metabolites and urea concentration at the Agricultural Experiment Station Chemical Laboratories (University of Missouri, Columbia).

Plasma from K_2EDTA tubes was used to analyze bovine growth hormone (**GH**). Individual samples were analyzed in triplicate and in a single RIA (Gorewit, 1981; Kobayashi et al., 1999). Recombinantly derived GH (CYT-636, Prospec-Tany Technogene Ltd., Ness-Ziona, Israel) was used as standard and iodinated trace. Trace GH was iodinated with ^{125}I by the Wright Center of Innovation in Biomedical Imaging (Columbus, OH; details in Appendix). For the RIA, the first antibody (monkey anti-bovine GH; AFPB55; A. F. Parlow, National Hormone and Pituitary Program, Torrance, CA) was diluted 1:30,000 and the second antibody (goat anti-monkey IgG; lot #413-13RR-05; Antibodies Inc., Davis, CA) was diluted 1:75. The minimum detectable concentration of GH was 0.5 ng/mL, and intra-assay coefficient of variation was 14.1%.

Statistical Analyses

One multiparous cow died from intestinal torsion (DMP at 49 DIM), and 2 primiparous cows sustained

Table 2. Nutrient composition of diets (DM basis) fed during treatment and carryover phases[1]

	Treatment[3]				
Nutrient[2]	DMP	AMP	Blend	Blend-fNDF	Carryover
DM, %	61.3	60.9	61.2	67.4	61.9
OM, %	92.4	92.0	92.4	92.6	92.8
CP, %	16.9	20.2	19.9	19.7	16.3
NDF_{om+cp}, %	30.2	27.7	28.7	28.3	29.9
Forage NDF, %	24.3	24.4	24.3	19.6	23.7
Starch, %	23.7	22.8	23.7	25.4	25.7
LCFA, %	3.08	3.24	3.79	3.74	4.50
NE_L,[4] Mcal/kg	1.63	1.68	1.64	1.66	1.64
Ca, %	1.06	1.23	1.09	1.05	1.08
P, %	0.39	0.44	0.48	0.45	0.41
Mg, %	0.34	0.35	0.37	0.38	0.41
K, %	1.95	2.10	1.94	1.80	1.75
Na, %	0.34	0.35	0.32	0.25	0.31
Cl, %	0.53	0.53	0.60	0.57	0.63
S, %	0.20	0.24	0.30	0.30	0.17
Cu, mg/kg	14	24	15	15	19
Mn, mg/kg	61	67	60	49	57
Zn, mg/kg	82	103	88	71	71
DCAD, mEq/kg	374	392	280	202	295

[1]Cows were fed 1 of 4 treatment diets immediately after calving until 25 DIM. During the carryover phase (25–92 DIM), all cows were fed the carryover diet from 25 to 50 DIM in tiestalls.

[2]NDF_{om+cp} = NDF − neutral detergent insoluble (NDI) CP − NDI ash; LCFA = long-chain fatty acids; DCAD (mEq/kg) = Na + K − Cl − S.

[3]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF).

[4]Estimated using the NRC (2001) model with treatment-average DMI.

physical injuries requiring euthanasia (Blend-fNDF at 85 DIM and AMP at 63 DIM). Available data from these cows were included in analyses.

Data were analyzed using PROC MIXED (v9.4, SAS Institute Inc., Cary, NC). Treatment and carryover phases were analyzed separately. Average DMI and milk production were calculated for each week within a phase. Postpartum models included the fixed effects of treatment, parity, time (DIM or week of lactation; repeated), all 2- and 3-way interactions, and the random effects of block nested within parity and residual error. For repeated measures, covariance structures were chosen based on lowest Bayesian information criterion. The autoregressive covariance structure was used for production data and heterogeneous compound symmetry for blood and milk FA profile. Cumulative yields of milk and milk components from 0 to 92 DIM were calculated and analyzed using a model including the fixed effects of treatment, parity and their interaction, and the random effects of block nested within parity and residual error. Denominator degrees of freedom for all models were adjusted using the Kenward-Roger option. Orthogonal contrasts were made a priori to evaluate the effect of MP concentration (DMP vs. AMP + Blend), AA profile (AMP vs. Blend) and fNDF concentration (Blend vs. Blend-fNDF). The 3 contrasts and 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 within treatment or treatment-parity followed by a Fisher least significant difference (LSD) test to separate means.

The time variable was week for production data and DIM for plasma metabolites and milk FA profile. Because of their dynamic nature during the fresh period, milk yield and DMI during the treatment phase were analyzed with time as DIM. Somatic cell count was $\log_{10}$ transformed for analysis and was not back-transformed. Plasma GH, FA, BHB, and 3-methyl-His were non-normally distributed and were natural log-transformed for analysis, followed by back transformation of means and standard error of means (**SEM**) for data tables (Jørgensen and Pedersen, 1998). Because of missing data, the highest SEM are reported for each dependent variable within a parity.

RESULTS AND DISCUSSION

Prepartum Phase

The prepartum diet had lower CP than formulated (11.5 vs. 14% CP; Tebbe, 2020) because grass hay and silage had lower CP concentrations than expected (hay: 9.4 vs. 13.0% and silage: 10.8 vs. 13.0% CP). Based on DMI during the prepartum phase (primigravid heifers: 10.7 kg of DMI/d; multigravid cows: 10.6 kg of DMI/d), predicted MP balance (NRC, 2001) from −14 to 0 d relative to calving was ~780 g/d for both parities. This MP intake was ~30 and 2.5% below intakes shown to maximize milk protein yields in early lactation for primigravid and multigravid Holsteins (1,100 and 800 g of MP/d, respectively) based on a meta-analysis (Husnain and Santos, 2019). Prepartum protein concentration may affect postpartum production responses (Amirabadi Farahani et al., 2019).

Fresh Cow Treatment Diets

The DMP diet met 87% of MP requirements (NRC, 2001), whereas AMP, Blend, and Blend-fNDF met 104, 110, and 111% of MP requirements, respectively (Table 3). The DMP diet had Lys, Met, and His concentrations near recommendations (6.8% Lys and 2.3% Met of MP, Schwab et al., 2009; 2.3% His of MP, Lee et al., 2012a). Increasing RUP primarily with soy in the AMP caused AA imbalances, and Lys, Met, and His (% of MP) were 9, 13, and 5% below recommendations, respectively. Conversely, Blend and Blend-fNDF essentially met Lys, Met, and His recommendations. The main difference between Blend-fNDF and Blend was 20% less fNDF, but they had similar NDF concentrations (average 31.0% NDF). The DCAD concentration was about 80 mEq/kg lower and starch 1.7% DM greater for Blend-fNDF versus Blend. The standard deviations (**SD**) for nutrient concentrations in experimental diets were calculated (Tebbe, 2020). Average monthly SD for CP and fNDF was similar across treatment diets (0.31 and 0.31% of DM, respectively).

Production During Treatment

From 1 to 25 DIM, no treatment by time interactions were found for DMI ($P = 0.96$, Figure 1). Intake did not increase with increasing MP concentration, but DMI was increased about 1.1 kg/d across both parity for Blend versus AMP ($P = 0.01$, Table 4). Carder and Weiss (2017) found similar DMI from 3 to 21 DIM when MP increased from 85 to 95% of requirements (16.5 vs. 18.5% CP) or for a diet meeting 95% of requirements and balanced for Lys and Met (17.5% CP); however, the AA balanced diet in this study was compared isonitrogenously. Balancing AA supply with RP-His, RP-Lys, and RP-Met increased DMI 7% in mid lactation cows fed isonitrogenous and MP-deficient diets (Giallongo et al., 2016). Based on samples from in situ incubation (Tebbe and Weiss, 2021), the Blend

treatment had about 2% (DM) more RDP than the AMP treatment, which can also increase DM digestibility and DMI (Lee et al., 2012b).

No treatment by day interactions were found for milk yield ($P = 0.99$; Figure 1). Concentrations of milk components were similar ($P \geq 0.14$) among treatments, whereas yields of milk, ECM, and milk components all increased ($P \leq 0.06$) 6 to 7% across parity with greater MP concentrations. Using a mixture of protein sources to linearly increase MP concentration via RUP incrementally increased milk and milk component yields during the first 21 DIM (Amanlou et al., 2017). Dietary nitrogen use efficiency (**NUE**, % = milk true protein-N ÷ N intake) was less ($P = 0.01$) with greater MP and was less in multiparous versus primiparous cows (treatment average 34.0 vs. 30.3%; parity: $P < 0.01$). Greater NUE would partially be from more skeletal muscle catabolism (see below) and empty-body CP mobilization (Tebbe and Weiss, 2021). Yields of milk and milk components ($P \geq 0.25$) and NUE ($P = 0.22$) were similar between Blend and AMP.

Concentration of fNDF interacted with parity to affect DMI ($P = 0.05$). In multiparous cows, DMI decreased for Blend-fNDF versus Blend, whereas DMI increased in primiparous cows. As we observed with primiparous cows, replacing fNDF with nonforage NDF often increases DMI (Allen, 2000). However, Blend-fNDF would have less effective NDF and Blend-fNDF had lower DCAD and greater starch concentrations compared with Blend. Interactions on DMI could be related to greater fermentability in Blend-fNDF and susceptibility of acidosis in multiparous versus primiparous cows (Maekawa et al., 2002), which is partially supported by the data on milk FA profile (see below). Less effective fiber (Swain and Armentano, 1994), lower DCAD (Iwaniuk and Erdman, 2015), and more fermentable starch have been related to lower DMI (Allen et al., 2009).

Similar to DMI, effects of fNDF interacted ($P \leq 0.02$) with parity on milk production. Milk yield was lower for Blend-fNDF versus Blend in multiparous cows but greater in primiparous cows. Lower fNDF had no effect on concentration of milk components ($P \geq 0.19$). Thus, similar interactions of fNDF by parity were found for ECM and fat yields: multiparous cows decreased yields with lower fNDF, whereas primiparous cows increased yields. There were no interactions or main effects of fNDF on milk protein and lactose yields. Lower milk fat yield with less fNDF could be caused by less effective fiber (Swain and Armentano, 1994) or lower DCAD concentration (Iwaniuk and Erdman, 2015). Less fNDF increased losses of BCS and empty body energy in both parities (Tebbe and Weiss, 2021), suggesting that energy intake was lower and could also be causing lower milk and milk fat yields.

A treatment × week interaction was found for MUN ($P = 0.08$; data not shown) but no treatment × parity interactions ($P \geq 0.63$) were observed. At wk 1, MUN was unaffected by MP concentration (average 15.3 mg/dL) but increased over time. At the end of treatment, MUN was greater for Blend and AMP (average 17.3 mg/dL) compared with DMP (14.7 mg/dL). However, MUN for cows fed Blend-fNDF decreased over time and was less at wk 1 compared with Blend (17.1 vs. 15.8

Table 3. Protein balances and AA profile of treatments[1]

Item	Treatment[2]			
	DMP	AMP	Blend	Blend-fNDF
MP supply, g/d	1,856	2,329	2,471	2,482
MP requirements, g/d	2,127	2,237	2,247	2,227
MP balance, g/d	−282	93	224	255
Digestible AA supply,[3] % of MP				
Arginine	4.84	4.86	4.64	4.65
Histidine	2.25	2.18	2.30	2.32
Isoleucine	4.86	4.74	4.62	4.61
Leucine	8.64	8.85	9.06	9.05
Lysine	6.61	6.16	6.62	6.62
Methionine	2.39	2.01	2.31	2.31
Phenylalanine	5.06	5.14	5.06	5.06
Threonine	4.79	4.64	4.55	4.54
Valine	5.45	5.40	5.34	5.33

[1]Cows were fed 1 of 4 treatment diets immediately after calving until 25 DIM. Balances and supply were calculated using NRC (2001) and average DMI, milk production, and milk components from the treatment phase.

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF).

[3]Amino acid concentrations of diets from values in NRC (2001).

mg/dL) and lower for Blend-fNDF once treatments ended (15.3 vs. 17.4 mg/dL). The MUN was similar ($P \geq 0.16$) over time for Blend and AMP.

Blood Metabolites

Treatment interactions with parity and DIM were not detected for any plasma metabolites (Table 5; $P \geq 0.12$). Plasma albumin increased with DIM ($P = 0.01$) and MP concentrations ($P = 0.04$). Increased plasma albumin concentration with greater MP concentration agrees with Amanlou et al. (2017) and is likely a result of increased hepatic synthesis of albumin (Larsen et al., 2017).

Plasma concentrations of GH were decreased when MP concentration ($P = 0.03$) and DIM increased ($P = 0.01$). No effect of AA profile or fNDF concentration was found ($P \geq 0.19$). Greater GH is associated with greater partitioning of nutrients and mobilization of body tissues for lactation, which diminishes as DIM increases (Bauman and Currie, 1980). Lower GH was found 1 wk before parturition in dairy cows fed 80 versus 100% of CP requirements (Chew et al., 1984). Lower GH with greater MP concentration may indicate less mobilization of body protein, which is supported by results for plasma 3-methyl-His (see below). Concentration of GH also decreased more for primiparous cows as DIM increased and was lower at 25 DIM in primiparous

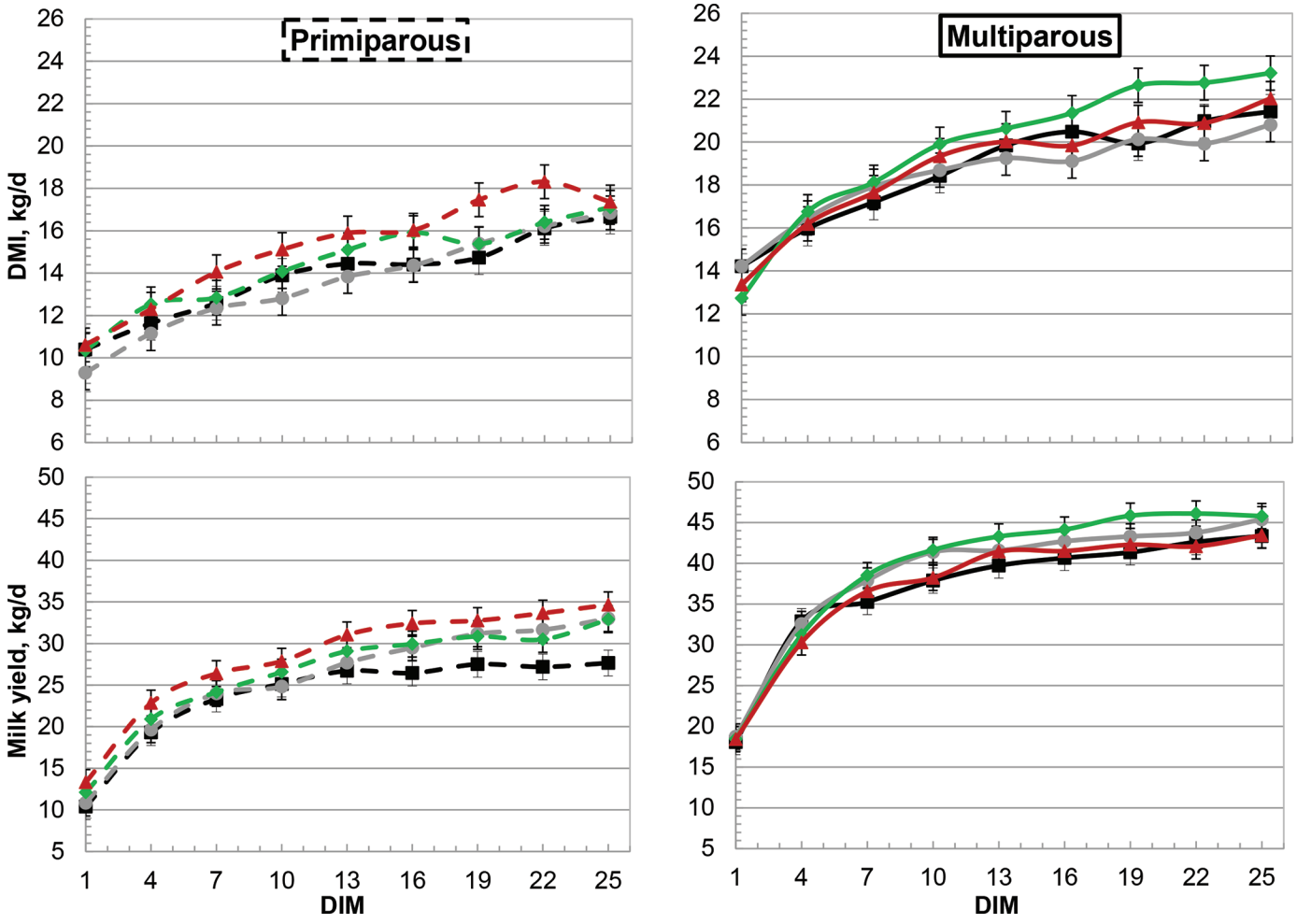

Figure 1. Effects of feeding diets with different concentrations of MP, AA profile, or forage NDF (fNDF) concentrations on DMI (top row) and milk yield (bottom row) during the first 25 DIM in primiparous (left) and multiparous (right) cows. Treatment diets were deficient MP (DMP; black squares; 16.9% 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 Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; red triangles; 19.8% CP). Days in milk, parity, and parity × treatment were significant ($P < 0.05$) but no effects of DIM × treatment ($P \geq 0.96$) or parity × DIM × treatment ($P \geq 0.91$) were found. Error bars indicate the standard error of the mean (DMI = 0.79 kg/d; milk yield = 1.55 kg/d), and the average of every third day is shown.

Table 4. Effects of feeding fresh cows diets with high RUP and replacing either forage or nonforage NDF on intake and production during the treatment period (1 to 25 DIM)

		Treatment[2]					*P*-value[3]		
Item	Parity[1]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
DMI, kg/d[a]	P	13.8	13.7	14.6[x]	15.3[y]	0.47	0.31	0.01	0.83
	M	19.0	18.6	20.1[y]	19.2[x]				
Milk, kg/d[a]	P	24.3	26.3	26.7[x]	28.8[y]	1.04	0.01	0.51	0.81
	M	38.0	39.5	40.3[y]	37.7[x]				
ECM,[4] kg/d[a]	P	29.4	31.4	31.4[x]	32.9[y]	1.68	0.03	0.42	0.17
	M	46.1	48.7	51.1[y]	45.3[x]				
Milk fat, %	P	4.49	4.46	4.26	4.18	0.17	0.63	0.90	0.19
	M	4.58	4.72	4.96	4.62				
Milk protein, %	P	3.20	3.31	3.14	3.15	0.07	0.94	0.14	0.87
	M	3.26	3.24	3.23	3.24				
Milk lactose, %	P	4.83	4.87	4.87	4.91	0.03	0.43	0.86	0.27
	M	4.76	4.76	4.78	4.81				
Milk fat, kg/d[a]	P	1.17	1.21	1.21[x]	1.26[y]	0.08	0.04	0.25	0.06
	M	1.82	1.95	2.12[y]	1.79[x]				
Milk protein, kg/d	P	0.82	0.91	0.89	0.94	0.05	0.06	0.99	0.73
	M	1.29	1.34	1.37	1.28				
Milk lactose, kg/d	P	1.26	1.37	1.39	1.50	0.07	0.03	0.58	0.82
	M	1.91	2.00	2.05	1.91				
MUN, mg/dL[b]	P	14.2	15.5	16.4	15.2	0.85	0.01	0.27	0.19
	M	13.8	16.6	17.3	16.5				
NUE,[5] %	P	32.0	30.6	29.0	29.5	0.01	0.01	0.22	0.66
	M	38.0	33.3	32.2	32.7				
SCC, $\log_{10}$/mL $\times 10^3$	P	2.21	2.09	1.86	1.79	0.14	0.15	0.51	0.94
	M	1.48	1.33	1.38	1.43				

[a]Parity × treatment: $P < 0.10$.

[b]Week × treatment: $P < 0.10$.

[x,y]Average values for a treatment and parity in the same row followed by different superscripts differ ($P < 0.05$). Superscripts are only displayed for significant ($P < 0.10$) contrast (see footnote 3) by parity interactions.

[1]P = primiparous; M = multiparous.

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF).

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

[4]ECM, kg/d = 0.327 milk yield + 12.95 fat yield + 7.65 protein yield (Tyrrell and Reid, 1965).

[5]Dietary nitrogen use efficiency, % = milk true protein-N/N intake × 100.

Table 5. Effects of feeding fresh cows diets with high RUP and replacing either forage or nonforage NDF on plasma metabolite concentrations

		Treatment[2]					*P*-value[3]		
Metabolite	Parity[1]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
Albumin, g/dL	P	4.93	5.08	5.04	5.01	0.07	0.04	0.34	0.79
	M	5.01	5.10	5.05	5.10				
Growth hormone,[4] ng/mL	P	12.0	8.4	9.2	10.7	1.93	0.03	0.95	0.19
	M	11.8	9.4	8.8	11.3				
Creatinine, mg/dL	P	1.98	1.93	1.95	1.88	0.07	0.30	0.85	0.71
	M	2.08	2.00	2.01	2.03				
Glucose, mg/dL	P	65.4	67.1	68.2	67.2	1.30	0.42	0.68	0.95
	M	58.9	59.3	57.2	58.4				
FA,[4] μEq/L	P	490	547	512	484	50.3	0.18	0.82	0.19
	M	600	679	696	579				
BHB,[4] μmol/L	P	582	741	663	614	76.0	0.28	0.34	0.76
	M	765	802	739	750				

[1]P = primiparous; M = multiparous.

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF). Blood samples for plasma were drawn at 4, 7, 10, and 25 DIM and values are averaged over time. No time or parity interactions with treatment were found ($P > 0.10$).

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

[4]Natural log-transformed for data analysis.

compared with multiparous cows (6.1 vs. 8.4 ng/mL; parity × day, $P = 0.03$). Parity had no effect on GH concentration at 42 DIM (Bonczek et al., 1988).

Plasma creatinine was similar among treatments and parity but decreased from 4 to 25 DIM (2.07 vs. 1.89 mg/dL; day, $P < 0.01$). Parity and the interaction of day by parity were not significant ($P \geq 0.11$). Decreasing creatinine concentration with increasing DIM but no parity effect or parity by DIM interaction agrees with Megahed et al. (2019). Plasma creatinine is positively correlated with muscle mass; however, the lack of parity and time interactions for plasma creatinine does not align with GH and 3-methyl-His (see below) or to treatment effects on empty body CP (Tebbe and Weiss, 2021). This suggests creatinine may not be a sensitive indicator of muscle mobilization.

Plasma glucose, FA, and BHB concentrations were similar among treatments, and expected day and parity effects were found ($P \leq 0.05$; Grummer, 1995). During the first 10 DIM, the incidence rate for subclinical ketosis was 13.3% using a threshold of >1,200 μmol of BHB/L of plasma (Ospina et al., 2010). Incidence rates within parity-treatment groups ranged from 4 to 20% and, based on chi-squared analysis, were not affected by parity ($P = 0.21$), treatment ($P = 0.61$), or treatment by parity interactions ($P \geq 0.52$).

Plasma AA Metabolites and Urea Concentrations

Plasma concentrations of several EAA (Table 6) and NEAA (Table 7) were increased with greater MP concentrations ($P \leq 0.04$). However, EAA including Arg, Ile, and Val were increased mainly from AMP (AA profile: $P \leq 0.05$), suggesting that those AA were in excess when using RUP primarily from soy. Plasma Orn (Table 8), an AA intermediate in the urea cycle, also increased for AMP, which is additional evidence of excess AA-N being catabolized and used for ureagenesis. Plasma Cit and urea-N were increased with greater MP concentrations ($P = 0.01$) but were unaffected by AA profile and fNDF ($P \geq 0.40$). Plasma Lys was greater in AMP versus Blend (AA profile $P = 0.01$), suggesting that Lys was first limiting for Blend. This occurred despite supplementing Lys in Blend versus AMP.

Less fNDF increased ($P < 0.05$) plasma concentrations of Leu, Phe, Asn, and Pro, and tended to increase ($P < 0.10$) Ile, Ala, and Tyr. Greater concentrations of several EAA but a similar milk protein yield for

Table 6. Effects of feeding fresh cows diets with high RUP and replacing either forage or nonforage NDF on plasma EAA

		Treatment[2]					*P*-value[3]		
EAA, μmol/L	Parity[1]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
Arginine	P	54.5	63.7	54.5	55.7	3.40	0.02	0.01	0.31
	M	50.3	66.0	51.9	57.1				
Histidine[b]	P	43.6	50.7	50.6	55.7	2.84	0.01	0.81	0.23
	M	46.1	52.9	51.7	53.2				
Isoleucine	P	88.7	121.8	86.9	99.2	8.55	0.01	0.01	0.07
	M	90.3	130.4	91.0	106.0				
Leucine	P	96.9	138.9	137.1	150.3	10.5	0.01	0.36	0.03
	M	103	158	141	172				
Lysine	P	62.8	67.9	53.2	61.2	4.25	0.68	0.01	0.23
	M	65.2	77.5	63.3	65.2				
Methionine[a]	P	36.2	26.5	43.0	45.0	2.90	0.71	0.01	0.09
	M	30.2	26.3	40.9	48.7				
Phenylalanine	P	40.5	46.1	44.9	49.3	2.20	0.01	0.15	0.04
	M	37.9	45.1	41.8	46.4				
Threonine	P	92.0	81.2	89.9	93.3	8.06	0.58	0.66	0.28
	M	97.3	97.6	95.6	108.5				
Tryptophan	P	54.3	54.5	57.2	56.4	1.98	0.48	0.29	0.51
	M	53.9	57.7	51.2	54.4				
Valine	P	169	235	191	206	18.2	0.01	0.01	0.14
	M	181	269	199	237				
EAA	P	739	888	809	872	53.2	0.01	0.05	0.13
	M	755	981	865	951				

[a]Day × treatment: $P < 0.10$.

[b]Parity × day × treatment: $P < 0.15$.

[1]P = primiparous; M = multiparous.

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF). Plasma samples were taken at 7 and 25 DIM in 16 random blocks of cows (8 blocks primiparous; 8 multiparous) and values are averaged over time.

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

Blend-fNDF versus Blend suggested excess AA supply. A tendency for increased Orn was also found for Blend-fNDF versus Blend ($P = 0.08$). Interactions with parity or DIM were not found ($P \geq 0.15$) for the above plasma AA concentrations.

A DIM by treatment interaction was found for plasma Met ($P = 0.01$; Figure 2a) but no parity by treatment interactions were observed ($P \geq 0.34$). At 7 DIM, plasma Met was similar between DMP and AMP but was greater in Blend and unaffected by fNDF. At 25 DIM, plasma Met remained greater for Blend compared with AMP but was similar to that of DMP, and Blend-fNDF was greater than Blend. Lower plasma Met for AMP versus Blend agrees with deficiency of supply versus recommendations. Increasing plasma Met from 7 to 25 DIM for DMP suggests that high concentrations of supplemental Met may be needed very early in lactation when DMI is low. Increased Met from 7 to 25 DIM with less fNDF partially agrees with the greater plasma concentrations of other EAA and indicates excess Met supply.

In addition to milk protein synthesis, Met is also used as a methyl donor (Nelson and Cox, 2018) and for antioxidant synthesis (i.e., taurine and glutathione). The Met used for methyl donation and not remethylated or used for antioxidant synthesis can increase homocysteine. In this study, homocysteine had interactions of parity with AA profile, fNDF concentration, and DIM ($P \leq 0.09$), but no parity by DIM by treatment interaction ($P = 0.98$). In multiparous cows, homocysteine was lower in Blend than in AMP or Blend-fNDF, whereas homocysteine was similar for AMP, Blend, and Blend-fNDF in primiparous cows (average of 4.91 μmol/L). Homocysteine, however, increased from 7 to 25 DIM in primiparous cows (4.62 vs. 5.45 μmol/L) but was similar between DIM for multiparous cows (average of 4.66 μmol/L; parity × DIM, $P = 0.01$). A similar parity by DIM interaction ($P = 0.01$) was found for sarcosine (7 vs. 25 DIM, primiparous: 2.68 vs. 7.33 μmol/L; multiparous: 6.56 vs. 0.79 μmol/L), an end-product of 3 methyl donations from betaine to homocysteine. Increased homocysteine and sarcosine over time in primiparous versus multiparous cows may suggest that primiparous cows require more methyl donors in very early lactation. Additional methyl donors for primiparous fresh cows is supported by Potts et al. (2020), who found that supplementing RP-Met improved milk fat yield in multiparous cows, whereas supplementing RP-choline, which can be synthesized to betaine and donate 3 methyl groups, improved milk yield in primiparous cows regardless of RP-Met supplementation.

A DIM by treatment interaction was found for plasma cystathionine ($P = 0.01$; Figure 2b). At 7 DIM, cystathionine concentration was greater in Blend than in AMP but similar to that of DMP and Blend-fNDF.

Table 7. Effects of feeding fresh cows diets with high RUP and replacing either forage or nonforage NDF on plasma NEAA

		Treatment[2]					*P*-value[3]		
NEAA, μmol/L	Parity[1]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
Alanine	P	240	223	229	232	15.9	0.77	0.52	0.06
	M	197	216	190	247				
Aspartate	P	5.67	7.93	6.84	6.61	0.78	0.12	0.15	0.82
	M	5.23	6.03	4.99	5.55				
Asparagine	P	37.1	41.8	38.6	40.5	2.88	0.04	0.04	0.04
	M	31.9	41.5	34.0	42.4				
Glutamate	P	56.0	60.4	59.1	62.0	2.33	0.21	0.23	0.32
	M	47.8	51.3	47.1	48.9				
Glutamine	P	271	276	271	281	13.0	0.39	0.36	0.25
	M	218	245	226	247				
Glycine	P	453	404	454	439	32.2	0.82	0.52	0.81
	M	495	513	501	502				
Proline	P	82.5	92.6	96.5	98.4	6.29	0.01	0.65	0.04
	M	73.6	94.2	84.8	108.7				
Serine	P	111	104	116	107	7.50	0.86	0.77	0.43
	M	98	105	98	118				
Tyrosine	P	34.1	39.4	43.1	43.3	3.12	0.01	0.84	0.06
	M	28.5	37.7	35.2	46.2				
NEAA	P	1,291	1,250	1,314	1,310	55.0	0.48	0.80	0.16
	M	1,196	13,310	1,221	1,366				

[1]P = primiparous; M = multiparous. No treatment × parity interactions were found ($P > 0.10$).

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF). Plasma samples were taken at 7 and 25 DIM in 16 random blocks of cows (8 blocks primiparous; 8 multiparous) and values are averaged over time.

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

By 25 DIM, cystathionine increased for all treatments; however, it was lower in AMP than in DMP, Blend, and Blend-fNDF (no effect of fNDF, $P = 0.18$). Taurine tended to have a similar type of interaction ($P = 0.15$; data not shown). Positive correlations between plasma Met and cystathionine (r = 0.59) and cystathionine and taurine (r = 0.58) were found ($P < 0.001$), which supports Zhou et al. (2017) and suggests Met supply above requirements for milk protein synthesis can be used for antioxidant production. No parity × treatment interactions were found for plasma cystathionine or taurine ($P \geq 0.15$). Lower homocysteine for multiparous versus primiparous cows fed a better AA supply combined with no interaction for taurine could mean more taurine was utilized or more homocysteine was remethylated into Met in multiparous cows.

A parity by DIM by treatment interaction was found for plasma His ($P = 0.01$; Figure 3a). At 7 DIM, plasma His was similar across treatments and parities but, at 25 DIM, was lower for DMP than for AMP and Blend. The decrease at 25 DIM for DMP was greater in primiparous cows and led to lower His concentrations compared with multiparous cows. Plasma concentrations of carnosine (also known as β-alanyl-L-histidine) also tended ($P = 0.06$) to be lower for DMP than for AMP and Blend, but no interactions of treatment with parity or DIM were found ($P \geq 0.15$). Plasma His and carnosine were unaffected by fNDF concentration ($P \geq 0.23$). In mid-lactation cows, deficient His supply decreases plasma concentrations of His and carnosine (Giallongo et al., 2017). Lower plasma His and carnosine for DMP compared with AMP and Blend suggests that the metabolizable His supplied by DMP became inadequate over time for fresh cows.

A tendency for a parity by DIM by treatment interaction was found for plasma 3-methyl-His ($P = 0.11$, Figure 3b). At 7 DIM, plasma 3-methyl-His was greater for DMP than for AMP and Blend, but at 25 DIM became similar across treatments. The decrease from 7 to 25 DIM was greater in multiparous than in primiparous cows. Plasma 3-methyl-His was unaffected by fNDF concentrations ($P = 0.30$). Increased plasma 3-methyl-His concentration has been found in wk 1 versus wk 4 of lactation and was increased in fresh

Table 8. Effects of feeding fresh cows diets with high RUP and replacing either forage or nonforage NDF on plasma AA metabolites, nonproteinogenic AA, and urea concentrations

		Treatment[2]					*P*-value[3]		
Metabolite, μmol/L	Parity[1]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
1-Methyl-L-His[a]	P	5.49	4.59^{x}	6.07^{x}	5.83	0.73	0.75	0.61	0.23
	M	6.27	7.85^{y}	5.71^{x}	7.52				
3-Methyl-L-His[4,c]	P	4.53	3.69	3.36	3.66	0.37	0.01	0.70	0.30
	M	6.06	4.70	4.84	5.25				
Carnosine	P	8.30	10.4	11.2	10.6	1.11	0.06	0.56	0.75
	M	9.61	10.6	11.0	12.3				
Citrulline	P	68.9	78.5	81.1	76.0	6.03	0.01	0.78	0.96
	M	94.2	106	100	105				
Cystathionine[b]	P	1.98	1.41	2.22	2.14	0.16	0.17	0.01	0.35
	M	2.01	1.45	2.10	2.49				
Homocystine[a]	P	5.41	4.51^{x}	5.34^{x}	4.87	0.39	0.88	0.71	0.60
	M	4.28	5.22^{y}	4.10^{x}	4.98				
Ornithine	P	25.5	30.7	27.5	30.9	2.33	0.02	0.01	0.08
	M	29.1	41.3	29.0	34.0				
Sarcosine[a]	P	6.56^{y}	3.95^{x}	4.55^{x}	4.97	0.64	0.02	0.10	0.93
	M	3.49^{x}	3.20^{x}	4.27^{x}	3.75				
Taurine	P	38.6	34.4	47.8	49.6	3.67	0.76	0.01	0.72
	M	52.1	42.7	60.4	61.2				
Urea-N, mg/dL	P	13.1	17.0	17.5	16.0	0.94	0.01	0.40	0.55
	M	15.0	17.4	17.6	18.2				

[a]Parity × treatment: $P < 0.10$.

[b]Day × treatment: $P < 0.10$.

[c]Parity × day × treatment: $P < 0.15$.

[x,y]Average values for a treatment and parity in the same row followed by different superscripts differ ($P < 0.05$). Superscripts are only displayed for significant ($P < 0.10$) contrast (see footnote 3) by parity interactions.

[1]P = primiparous; M = multiparous.

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF). Plasma samples were taken at 7 and 25 DIM in 16 random blocks of cows (8 blocks primiparous; 8 multiparous) and values are averaged over time.

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

[4]Natural log-transformed for data analysis.

cows fed 17% versus 19% CP (Sawada et al., 2013). Plasma 3-methyl-His is a biomarker for skeletal muscle breakdown; this interaction supports results found for GH and suggests primiparous versus multiparous cows mobilize less protein for lactation, as was found in this experiment (Tebbe and Weiss, 2021).

Milk Fatty Acid Profile

Treatment diets had similar FA concentrations (Tebbe, 2020). No treatment by time or parity interactions were found ($P \geq 0.10$) for milk FA profile (Table 9). Concentrations of several odd- and branched-chain FA and isomers of *trans* 18:1 FA tended to decrease with increasing MP concentrations ($P < 0.10$). Decreased odd- and branched-chain FA concentrations is consistent with a meta-analysis (Vlaeminck et al., 2006a) that found a negative correlation between diet CP and concentrations of these milk FA. Greater concentration but similar yield with a lower MP concentration also occurred for de novo FA. The concentration of 17:0 was lower ($P = 0.03$), that of *cis*-11 18:1 was greater ($P = 0.01$) and that of *iso* 14:0 tended to be greater ($P = 0.09$) in Blend versus AMP. Compared with Blend, Blend-fNDF tended to have decreased *iso* 14:0 ($P = 0.06$) and increased 18:2 ($P = 0.05$) and tended to have increased 18:3 ($P = 0.06$). The concentration of 17:0 is negatively associated with ruminal acetate and positively associated with propionate, whereas *iso* 14:0 shows the opposite pattern (Vlaeminck et al., 2006b). Decreased *iso* 14:0 with less fNDF supports the contention that Blend-fNDF had increased fermentability compared with Blend and could also explain lower milk

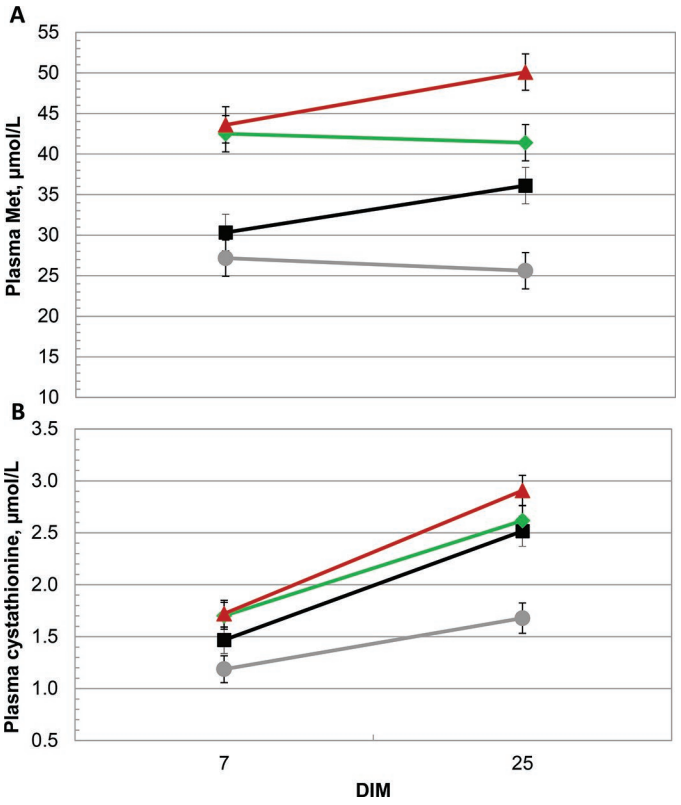

Figure 2. Effects of feeding diets with different concentrations of MP, AA profile, or forage NDF (fNDF) concentrations on plasma (A) Met and (B) cystathionine at 7 and 25 DIM. Treatment diets were deficient MP (DMP; black squares; 16.9% CP), adequate MP using primarily soy to increase RUP concentration (AMP; gray circles; 20.2% CP), adequate MP using a blend of RUP and AA sources (Blend; green diamonds; 19.9% CP), and Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; red triangles; 19.8% CP). Day × treatment interactions were found (Met, $P = 0.01$; cystathionine, $P = 0.01$). Error bars indicate the standard error of the mean (Met = 2.60 µmol/L; cystathionine = 0.15 µmol/L).

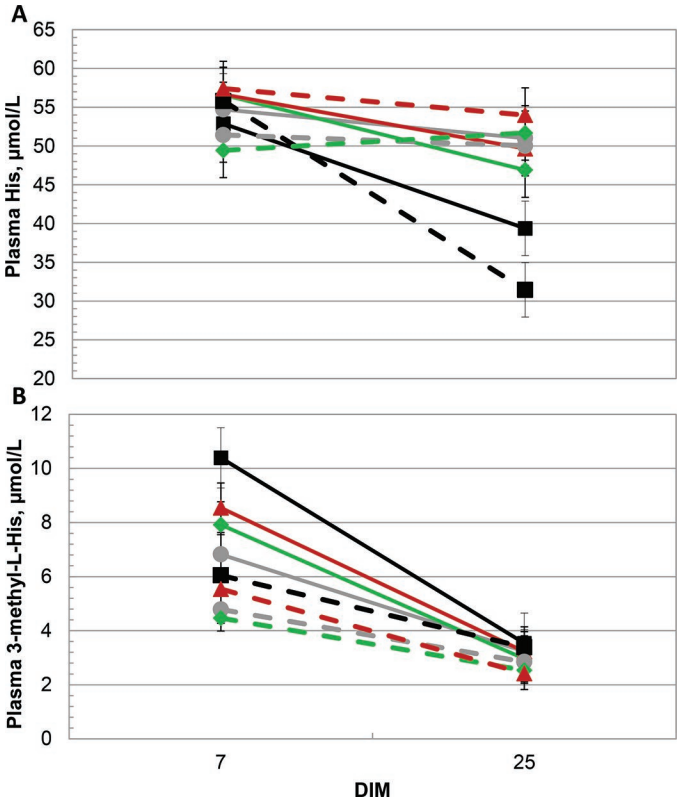

Figure 3. Effects of feeding diets with different concentrations of MP, AA profile, or forage NDF (fNDF) concentrations on plasma (A) His and (B) 3-methyl-L-His concentrations in primiparous (dashed lines) and multiparous (solid lines) cows at 7 and 25 DIM. Treatment diets were deficient MP (DMP; black 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). Parity × day × treatment interactions were found (His, $P = 0.01$; 3-methyl-L-His, $P = 0.11$). Error bars indicate the standard error of the mean (His = 2.84 µmol/L; 3-methyl-L-His = 1.1 µmol/L).

Table 9. Effects of feeding fresh cows diets with high RUP and replacing either forage or nonforage NDF on milk fatty acids (≤17C chain length)

	Treatment[2]					*P*-value[3]		
Fatty acid,[1] g/kg	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
SCFA[4]	216.2	214.2	217.6	218.6	9.3	0.98	0.77	0.93
iso 13:0	0.28	0.29	0.28	0.25	0.02	0.63	0.49	0.19
anteiso 13:0	0.31	0.28	0.29	0.33	0.03	0.49	0.67	0.30
iso 14:0	0.59	0.58	0.63	0.57	0.02	0.60	0.09	0.06
iso 15:0	1.78	1.60	1.67	1.66	0.05	0.02	0.38	0.90
anteiso 15:0	3.88	3.49	3.47	3.61	0.15	0.03	0.93	0.51
iso 16:0	1.95	1.90	1.99	1.88	0.07	0.98	0.36	0.29
16:0	274.0	262.6	257.2	260.9	3.9	0.01	0.29	0.46
iso 17:0	3.94	3.56	3.76	3.74	0.17	0.08	0.31	0.92
cis-9 16:1 + *anteiso* 17:0	20.6	21.5	21.9	21.6	0.9	0.26	0.70	0.78
17:0	7.86	7.89	7.46	7.66	0.15	0.25	0.03	0.28
17:1	3.51	3.70	3.67	3.65	0.20	0.40	0.89	0.93
18:0	131.8	132.4	129.4	128.3	2.9	0.97	0.41	0.75
trans-6 and *trans*-8 18:1	3.60	3.57	3.67	3.63	0.14	0.90	0.63	0.86
trans-9 18:1	2.54	2.42	2.59	2.60	0.08	0.71	0.12	0.90
trans-10 18:1	7.24	8.13	6.16	6.57	1.03	0.94	0.18	0.78
trans-11 18:1	16.7	14.8	15.5	15.1	0.67	0.04	0.44	0.66
trans-12 18:1	3.90	3.21	3.27	3.63	0.19	0.01	0.76	0.11
cis-9 18:1	257.8	270.8	275.9	270.4	9.9	0.17	0.74	0.72
cis-11 18:1	10.8	10.8	12.5	12.0	0.49	0.12	0.01	0.40
18:2	21.1	22.0	21.1	22.5	0.57	0.54	0.17	0.05
18:3	3.28	3.38	3.28	3.50	0.10	0.62	0.37	0.06
20:0	1.25	1.22	1.26	1.26	0.03	0.86	0.30	0.97
20:1	0.90	0.90	0.91	0.91	0.04	0.86	0.83	0.93
cis-9,*trans*-11 18:2	5.02	4.77	5.12	5.04	0.17	0.67	0.11	0.72
trans-10,*cis*-12 18:2	0.04	0.05	0.04	0.04	0.011	0.73	0.64	0.77
Other CLA	0.14	0.17	0.15	0.15	0.013	0.37	0.19	0.69

[1]Number of carbons: number of double bonds.

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF). Milk fatty acid profile measured at 7 and 25 DIM and averaged across parities and time. No time or parity interactions with treatment were found ($P > 0.10$).

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

[4]Short-chain fatty acids, g/kg = C 4:0 + 6:0 + 8:0 + 10:0 + 12:0 + 13:0 +14:0 + 15:0.

fat yield. Increased 18:2 and 18:3 without changes in 18:2 or 18:1 isomers may indicate more UFA escaping biohydrogenation for Blend-fNDF versus Blend.

Carryover Effects of Treatments

When cows received a common diet, DMI from 26 to 50 DIM remained unaffected by MP concentration fed during the fresh period ($P = 0.53$, Table 10). However, AA profile ($P = 0.09$) and fNDF ($P = 0.03$) interacted with parity to affect DMI. In multiparous cows, DMI remained increased for Blend compared with AMP and Blend-fNDF, whereas DMI became similar in primiparous cows for Blend compared with AMP and Blend-fNDF.

Fresh cow treatments carried over and affected milk yield measured from 26 to 50 DIM (Table 10) but not for the entire carryover period (26 to 92 DIM; Table 11). From 26 to 50 DIM, milk yield remained lower for Blend-fNDF versus Blend in multiparous cows and was similar in primiparous cows. Greater MP concentration during the fresh period also increased NUE from 26 to 50 DIM. Greater 3-methyl-His at 7 DIM and less NUE for milk production could be because cows fed DMP were replenishing losses of skeletal muscle during the carryover period.

For carryover effects measured during the entire carryover phase (Table 11), parity interacted with AA profile on milk fat percent and was greater for Blend versus AMP in multiparous cows but similar in primiparous cows. A carryover effect on milk fat percent for fresh cows fed a better AA profile agrees with Carder and Weiss (2017). Greater milk fat percent caused yields of milk fat and ECM (Figure 4) to remain increased in multiparous cows fed Blend versus AMP and Blend-fNDF, whereas yields of milk fat and ECM in primiparous cows were similar for Blend versus AMP and Blend-fNDF. Milk protein and lactose percent had no carryover effects, were unaffected by treatments ($P \geq 0.17$), and had no treatment by parity interactions

Table 10. Carryover effects of feeding fresh cows diets with high RUP and replacing either forage or nonforage NDF on intake and milk production (26 to 50 DIM)

		Treatment[2]					*P*-value[3]		
Item	Parity[1]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
DMI, kg/d[a]	P	17.8	17.3[x]	17.1[x]	18.1[x]	0.67	0.52	0.17	0.48
	M	22.3	21.2[x]	23.1[y]	21.3[x]				
Milk, kg/d[a]	P	30.1	33.1	32.6[x]	34.2[x]	1.45	0.26	0.50	0.51
	M	44.6	43.5	46.0[y]	42.5[x]				
Milk fat, kg/d[a]	P	1.19	1.29[x]	1.19[x]	1.31[x]	0.09	0.77	0.17	0.34
	M	1.82	1.64[x]	1.98[y]	1.69[x]				
Milk protein, kg/d	P	0.90	0.97	0.96	1.00	0.04	0.42	0.50	0.82
	M	1.27	1.23	1.30	1.24				
Milk lactose, kg/d	P	1.53	1.71	1.67	1.74	0.08	0.20	0.56	0.49
	M	2.19	2.15	2.27	2.10				
NUE,[4] %	P	30.0	32.8	32.7	32.6	1.06	0.07	0.45	0.36
	M	33.5	34.4	33.0	34.9				

[a]Parity × treatment: $P < 0.10$.

[x,y]Average values for a treatment and parity in the same row followed by different superscripts differ ($P < 0.05$). Superscripts are only displayed for significant ($P < 0.10$) contrast (see footnote 3) by parity interactions.

[1]P = primiparous; M = multiparous.

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF). No time interactions with treatment ($P < 0.10$).

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

[4]Dietary nitrogen use efficiency, % = milk true protein-N/N intake × 100.

Table 11. Effects of feeding fresh cows diets with high RUP and replacing either forage or nonforage NDF on intake and production during the carryover period (26 to 92 DIM)

		Treatment[2]					*P*-value[3]		
Item	Parity[1]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
Milk, kg/d	P	31.8	34.7	33.8	34.6	1.36	0.43	0.58	0.53
	M	45.5	43.7	46.1	43.5				
ECM,[4] kg/d[a]	P	33.2	35.7[x]	34.4[x]	35.3[x]	1.34	0.59	0.07	0.23
	M	47.1	43.5[x]	49.2[y]	45.4[x]				
Milk fat, %[a]	P	3.71	3.62[x]	3.58[x]	3.58	0.14	0.66	0.08	0.47
	M	3.73	3.51[x]	3.98[y]	3.81				
Milk protein, %	P	2.98	2.91	2.92	2.92	0.05	0.32	0.85	0.17
	M	2.86	2.87	2.85	2.95				
Milk lactose, %	P	4.98	4.99	4.99	4.99	0.03	0.86	0.42	0.39
	M	4.83	4.82	4.86	4.89				
Milk fat, kg/d[a]	P	1.20	1.28[x]	1.22[x]	1.25[x]	0.07	0.78	0.02	0.13
	M	1.71	1.52[x]	1.85[y]	1.65[x]				
Milk protein, kg/d[a]	P	0.96	1.03[x]	1.00[x]	1.02	0.03	0.69	0.49	0.84
	M	1.31	1.25[x]	1.32[y]	1.29				
Milk lactose, kg/d[a]	P	1.61	1.77[x]	1.70[x]	1.74	0.07	0.44	0.60	0.62
	M	2.22	2.12[x]	2.25[y]	2.15				
MUN, mg/dL	P	14.1	13.5	14.1	13.4	0.61	0.08	0.05	0.08
	M	15.0	13.4	14.6	13.7				
SCC, $\log_{10}$/mL $\times 10^3$	P	1.63	1.63	1.35	1.41	0.11	0.29	0.08	0.76
	M	1.28	1.27	1.16	1.16				

[a]Parity × treatment: $P < 0.10$.

[b]Week × treatment: $P < 0.10$.

[x,y]Average values for a treatment and parity in the same row followed by different superscripts differ ($P < 0.05$). Superscripts are only displayed for significant ($P < 0.10$) contrast (see footnote 3) by parity interactions.

[1]P = primiparous; M = multiparous.

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF).

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

[4]ECM, kg/d = 0.327 milk yield + 12.95 fat yield + 7.65 protein yield (Tyrrell and Reid, 1965).

($P \geq 0.21$). Yields of protein and lactose did carry over and were greater in multiparous cows fed Blend compared with AMP but were similar in primiparous cows (parity by AA profile: $P \leq 0.09$).

The SCC was low overall during the carryover period but tended to decrease ($P = 0.08$) for cows fed Blend versus AMP. A carryover effect for lower SCC postpartum has been found when prepartum cows are supplemented with RP-Lys and RP-Met (Lee et al., 2019).

By 50 DIM, plasma metabolites measured (Table 5) were similar across treatments (data not shown) with the exception of glucose, which had parity by AA profile ($P < 0.01$) and fNDF ($P < 0.06$) interactions. In multiparous cows, plasma glucose was decreased in Blend (60.2 mg/dL) compared with AMP and Blend (65.4 and 63.6 mg/dL) but was similar across the high MP treatments in primiparous cows (average 66.9 mg/dL). The MP concentration had no effect ($P = 0.44$) or interaction with parity ($P = 0.42$) on plasma glucose. Decreased plasma glucose is associated with improved insulin sensitivity (De Koster and Opsomer, 2013). Milk FA profile did not carry over and was similar across treatments at 50 DIM ($P \geq 0.10$; data not shown).

Cumulative Milk Production

Cumulative yields of milk ($P = 0.10$) and milk lactose ($P = 0.10$) had an fNDF by parity interaction and were decreased for Blend-fNDF versus Blend in multiparous cows, whereas they were increased in primiparous cows (Table 12). Cumulative yields of ECM and milk fat had parity by AA profile ($P \leq 0.03$) and fNDF ($P \leq 0.04$) interactions. In multiparous cows, those fed Blend produced 23 kg more milk fat than those fed AMP and 19 kg more milk fat than those fed Blend-fNDF. However, for primiparous cows, cumulative yields were similar for Blend versus AMP and Blend-fNDF. Cumulative milk protein yield had no treatment or treatment by parity interactions ($P \geq 0.15$).

CONCLUSIONS

Increasing MP supply via increased RUP concentrations improved milk production during the first 25 DIM but did not concomitantly increase DMI unless the AA profile of the RUP supply was balanced. A greater and balanced AA supply in mature cows carried over and led to greater DMI and ECM yields later into lacta-

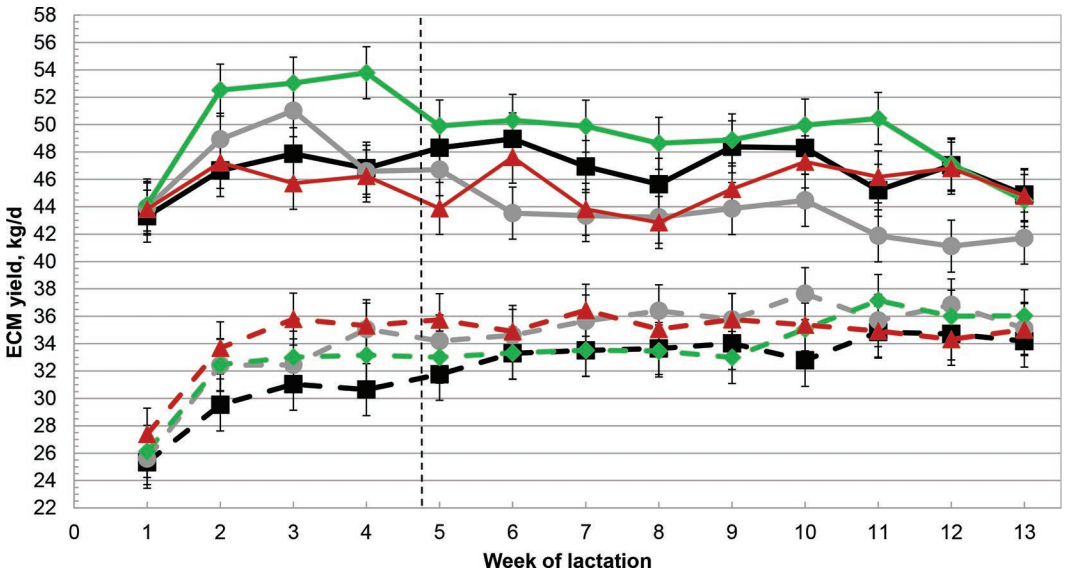

Figure 4. Concurrent and carryover effects of feeding diets with different concentrations of MP, AA profile, or forage NDF (fNDF) concentrations during the first 25 DIM (left side of vertical dashed line) on ECM yield in primiparous (dashed curves) and multiparous (solid curves) cows from 1 to 13 wk of lactation. 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 Blend replacing forage rather than nonforage NDF sources (Blend-fNDF; red triangles; 19.8% CP). Week, parity, and parity × treatment were significant ($P < 0.01$) but no effects of week × treatment ($P = 0.50$) or parity × week × treatment ($P = 0.13$) were found. Error bars indicate the standard error of the mean (1.90 kg/d).

Table 12. Effects of feeding fresh cows (0 to 25 DIM) diets with high RUP and replacing either forage or nonforage NDF on cumulative milk production from 0 to 92 DIM

		Treatment[2]					*P*-value[3]		
Item	Parity[1]	DMP	AMP	Blend	Blend-fNDF	SEM	MP	AA	fNDF
Milk, kg[a]	P	2,753	2,954	2,827[x]	3,042[y]	127	0.51	0.80	0.95
	M	3,993	3,903	4,092[y]	3,861[x]				
ECM, kg[a]	P	2,969	3,151[x]	2,986[x]	3,190[x]	139	0.58	0.36	0.54
	M	4,322	4,146[x]	4,548[y]	4,186[x]				
Milk fat, kg[a]	P	109	115[x]	108[x]	115[x]	6.8	0.65	0.19	0.29
	M	162	152[x]	175[y]	156[x]				
Milk protein, kg	P	86	90	86	92	3.6	0.74	0.91	0.78
	M	121	118	123	119				
Milk lactose, kg[a]	P	140	150	143[x]	154[y]	6.4	0.56	0.78	0.91
	M	197	191	201[y]	192[x]				

[a]Parity × treatment: $P < 0.10$.

[x,y]Average values for a treatment and parity in the same row followed by different superscripts differ ($P < 0.05$). Superscripts are only displayed for significant ($P < 0.10$) contrast (see footnote 3) by parity interactions.

[1]P = primiparous; M = multiparous.

[2]Treatments were deficient MP (DMP), adequate MP using primarily soy to increase RUP concentration (AMP), adequate MP using a blend of RUP and rumen-protected AA sources (Blend), and Blend replacing forage rather than nonforage NDF (Blend-fNDF).

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

tion. Positive outcomes from a greater and balanced AA supply to mature cows were not found when fNDF replaced RUP, indicating that mature cows have a higher fNDF requirement than primiparous cows when fed high MP diets in early lactation. Overall, these results demonstrate the importance of AA supply during the fresh period, especially on longer-term production in mature cows.

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APPENDIX

^{125}I-Labeling Protocol

Labeling of bovine growth hormone (GH) with ^{125}I was accomplished using a standard iodogen method (Fraker and Speck, 1978; Paus et al., 1982; Bailey, 1996). For a typical ^{125}I-labeling experiment, 0.050 mg of bovine GH was transferred into an iodogen tube (Pierce Biotechnology, Rockford, IL) containing 100 μL of phosphate buffer (0.1 *M*, pH 7.4), followed by the addition of a known amount (540 μCi) of ^{125}INa (Perkin Elmer Life Sciences, Waltham, MA). An additional 50 μL of phosphate buffer (0.1 *M*, pH 7.4), was then added to the mixture, which was covered with a lid and incubated at room temperature for 30 min with occasional swirling. The ^{125}I-labeled GH was loaded onto a Sephadex G-25 (PD-10) size-exclusion column (Thermo Fisher Scientific, Waltham, MA) and eluted with PBS for separation of ^{125}I-labeled bovine GH from the free iodide. Several fractions of 10 drops each were collected, and the fractions containing the highest radioactivity were combined in a preweighed plastic vial. The amount of radioactivity was determined using a dose calibrator. The percent yield of radiolabeling was calculated by dividing the total radioactivity of the combined sample by the amount of radioactivity added to the iodogen tube. Purity (>99%) of the ^{125}I-labeled GH sample was determined by a size-exclusion high-performance liquid chromatography method.