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Effect of source and frequency of rumen-protected protein supplementation on mammary gland amino acid metabolism and nitrogen balance of dairy cattle

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ABSTRACT

The AA profile of MP affects mammary gland metabolism and milk N efficiency of dairy cattle. Further, the frequency of dietary protein supplementation may influence N partitioning leading to reduced N excretion. This study investigated the effect of source and frequency of rumen-protected (RP) protein supplementation on apparent total-tract digestibility, milk production, mammary gland AA metabolism, and N balance of dairy cattle. Twenty-eight Holstein-Friesian cows (2.3 ± 0.9 lactations; 93 ± 27 DIM; mean ± SD) were used in a randomized complete block design and fed a basal TMR consisting of 41% corn silage, 32% grass silage, and 27% concentrate (DM basis) and formulated to meet 100% and 95% of net energy and MP requirements, respectively. Cows were adapted to the basal TMR in a freestall barn for 7 d, moved to individual tiestalls for 13 d of adaptation to dietary treatments, and then moved into climate respiration chambers for a 4-d measurement period. Treatments consisted of the basal TMR (CON; 159 g CP/kg DM) or the basal TMR including 1 of 3 iso-MP supplements: (1) 315-g mixture of RP soybean meal and RP rapeseed meal fed daily (ST-RPSR), (2) 384-g mixture of RP His, RP Lys, and RP Met fed daily (ST-RPAA), and (3) 768-g mixture of RP His, RP Lys, and RP Met fed every other day (OS-RPAA). The basal TMR with the addition of treatment supplements was designed to deliver 100% of required MP over a 48-h period. The mixture of His, Lys, and Met was formulated to deliver digestible AA in amounts relative to their concentration in casein. Compared with ST-RPSR, ST-RPAA increased milk protein and fat concentration, increased the arterial concentration of total His, Lys, and Met (HLM), decreased mammary clearance of HLM, and increased clearance of Phe, Leu, and Tyr (tendency for Leu and Tyr). Rumen-protected protein source did not affect N balance, but the marginal use efficiency (efficiency of transfer of RP protein supplement into milk protein) of ST-RPAA (67%) was higher than that of ST-RPSR (17%). Milk protein concentration decreased with OS-RPAA compared with ST-RPAA. Arterial concentration of HLM increased on the nonsupplemented day compared with the supplemented day with OS-RPAA, and there was no difference in arterial HLM concentration across days with ST-RPAA. Mammary uptake of HLM tended to increase on the nonsupplemented day compared with the supplemented day with OS-RPAA. Supplementation frequency of RP AA did not affect N balance or overall milk N efficiency, but the marginal use efficiency of OS-RPAA (49%) was lower compared with ST-RPAA. Overall, mammary glands responded to an increased supply of His, Lys, and Met by reducing efflux of other EAA when RP His, RP Lys, and RP Met were supplemented compared with RP plant proteins. Mammary glands increased sequestration of EAA (primarily HLM) on the nonsupplemented day with OS-RPAA, but supplementing RP AA according to a 24-h oscillating pattern did not increase N efficiency over static supplementation.

Key words: amino acid profile, milk nitrogen efficiency, oscillation, rumen-protected amino acid

INTRODUCTION

Protein-rich ingredients that contribute relatively more RUP compared with RDP are commonly included in dairy cattle diets to complement the AA profile of MP from microbial protein and increase total MP supply. The digestible AA profile from microbial protein and RUP can influence the marginal efficiency with which supplemental protein is transformed into milk protein (Haque et al., 2012; Nichols et al., 2019a; Yoder et al., 2020). Protein-rich ingredients such as soybean meal and rapeseed meal that have been chemically treated to

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

reduce their ruminal degradability (referred to in this current work as rumen-protected [**RP**] soybean meal or RP rapeseed meal) or byproducts that are low in ruminal protein degradability such as distillers grains, brewers grains, and corn gluten meal are commonly fed to increase MP supply. However, digestible AA delivered by these sources may result in limitations or redundancies with respect to efficient milk protein synthesis. For example, mammary glands prioritize use of EAA to synthesize NEAA (Doepel and Lapierre, 2010), but delivery of digestible NEAA is unavoidable when supplementing whole protein sources. Further, Met, Lys, and His are important for milk protein synthesis (e.g., Met and His maintain a 1:1 ratio of mammary uptake to milk protein output, and excess mammary uptake of Lys contributes N for NEAA synthesis; Lapierre et al., 2009, 2012; Nichols et al., 2022). Further, digestible supply of Lys and Met may be limited when feeding common protein-rich ingredients, particularly byproduct feeds (e.g., brewers grains, distillers grains, blood meal; Santos et al., 1998; Schwab and Broderick, 2017), and low His concentration in microbial protein may limit its supply when dietary CP content is reduced (Lapierre et al., 2021; Räisänen et al., 2022). These limitations, together with shifts toward precision feeding, have motivated research into supplementing commercially produced RP His, Lys, and Met to complement the digestible AA profile of MP. Studies have reported increases in milk protein concentration, milk protein yield, or milk N efficiency when supplementing RP His, Lys, and Met to diets deficient in total MP (Lee et al., 2012; Giallongo et al., 2016; Zang et al., 2021), or when combining plant protein supplements with RP AA (Nichols et al., 1998; Cabrita et al., 2011; Pereira et al., 2020), whereas some report no effect of RP AA supplementation (Liu et al., 2000; Stevens et al., 2021; Van den Bossche et al., 2023). Few studies have characterized the metabolic response of mammary glands to dietary supplementation with plant protein sources (Seymour et al., 1990; Bach et al., 2000; Pereira et al., 2020), and none have determined how this compares to supplementation with exclusively RP AA.

In growing ruminants, feeding protein supplements infrequently or offering diets that oscillate in dietary CP concentration over time compared with a constant dietary CP concentration has been shown to increase N retention and fiber digestibility in some studies (Archibeque et al., 2007; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011), but not all (Ludden et al., 2002; Cole et al., 2003). Positive effects during CP oscillations may be due in part to shifts in N metabolism when stimulating the use of recycled urea for microbial protein synthesis during periods of low-CP feeding, and subsequently replenishing N supply to the animal during periods of high-CP feeding. In dairy cattle, oscillating dietary CP content from low (ranging from 11.9% to 13.8%, DM basis) to high (ranging from 15.5% to 17.3%, DM basis) over phases of 24 or 48 h resulted in similar milk protein production and N efficiency relative to static CP feeding (Tebbe and Weiss, 2020; Rauch et al., 2021; Erickson et al., 2023), demonstrating the flexibility of digestive, absorptive, and metabolic processes in lactating dairy cattle under varying dietary CP supplies. In these studies, CP content was altered by shifting the ingredient composition of the concentrate portion of diets. To our knowledge, oscillating the supplementation of exclusively RP AA has never been investigated in dairy cattle. Further, the mammary gland metabolic response to intentional daily variations in digestible AA supply has never been characterized.

The current study had 2 main objectives. The first objective was to determine the effect of AA profile of supplemental MP from RP plant proteins or RP AA (specifically His, Met and Lys in a casein profile) on nutrient digestibility, milk production, mammary gland AA metabolism, and N balance. The second objective was to determine the effect of supplementation frequency of RP AA on the same parameters. We hypothesized that intramammary metabolism would respond differently to an AA profile from RP plant proteins versus RP His, Lys, and Met, and that MP from RP AA would be used with a greater efficiency than MP from plant protein sources. Furthermore, despite providing the same EAA profile, we hypothesized that differences in supply of absorbed EAA according to the oscillating frequency would result in different metabolic adaptations by the mammary glands.

MATERIALS AND METHODS

Experimental Design, Diet, and Treatments

This experiment was conducted from August through October 2020 at the animal research facilities of Wageningen University and Research (Wageningen, the Netherlands) under the Dutch Law on Animal Experiments in accordance with EU Directive 2010/63. All experimental procedures were approved by the Central Committee of Animal Experiments (The Hague, the Netherlands; 2017.D-0079.004).

Twenty-eight Holstein-Friesian dairy cows (93 ± 27 DIM; 2.3 ± 0.9 lactations; mean ± SD; 5 primiparous, 23 multiparous) were used in a randomized complete block design where cows were blocked by DIM and parity. Cows were fed a basal TMR throughout the entire study consisting of 40.7% corn silage, 32.0% grass silage, and 27.3% concentrate on a DM basis (Table 1), formulated to meet 100% and 95% of NE_L and MP requirements (CVB, 2018), respectively, for cows consuming 22 kg DM/d and producing 34 kg/d of milk containing 40 g/kg fat and 34 g/kg protein. Cows had individual and free ac-

Table 1. Analyzed and calculated chemical composition of the ingredients (corn silage, grass silage, and concentrate), rumen-protected protein supplements, and TMR (g/kg DM, unless otherwise noted)

	Ingredient			Supplement[1]		TMR[2]		
Item	Corn silage	Grass silage[3]	Concentrate[4]	RPSR	RPAA	CON	RPSR	RPAA
Analyzed								
DM, g/kg	320	470	897	877	977	450	452	453
Gross energy, MJ/kg DM	19.1	19.2	17.2	18.9	28.3	18.6	18.6	18.8
Crude ash	33	81	121	82	1	70	70	69
CP	85	142	288	431	551	159	162	165
Crude fat	29	26	30	29	431	28	28	35
NDF	399	548	161	204	NA[5]	382	380	376
ADF	227	304	70	179	NA	209	209	205
ADL	15	29	5	8	NA	17	16	16
Starch	324	NA	263	8	NA	204	201	200
Sugar	NA	72	84	92	NA	46	46	45
Calculated								
DVE[6]	55	50	170	367	263	85	88	88
OEB[7]	−36	23	68	17	24	11	11	11
NE_L,[8] MJ/kg DM	6.69	5.67	7.40	7.06	13.0	6.56	6.56	6.66

[1]RPSR = mixture of rumen-protected soybean meal and rumen-protected rapeseed meal with iso-MP contributions from both sources (MervoBest; Agrifirm, the Netherlands); RPAA = mixture of rumen-protected His (Ajinomoto Co., Japan), rumen-protected Lys (AjiPro-L; Ajinomoto Health & Nutrition), and rumen-protected Met (Smartamine M; Adisseo, France) in the profile of casein.

[2]Values for TMR were calculated based on ration composition and analyzed and calculated values obtained for forages, concentrate, and supplements. CON = basal TMR with no supplement; contained (g/kg DM): corn silage, 407; grass silage, 320; concentrate, 273. RPSR = basal TMR plus RPSR supplement; contained (g/kg DM): corn silage, 403; grass silage, 316; concentrate, 268; RPSR supplement, 12. RPAA = basal TMR plus RPAA supplement; contained (g/kg DM): corn silage, 401; grass silage, 315; concentrate 267; RPAA supplement, 17.

[3]Composed of diploid perennial ryegrass (*Lolium perenne*; approximately 70%) and timothy (*Phleum pratense*; approximately 30%).

[4]Contained (g/kg DM): ground corn 8% CP, 351; soybean meal 46% CP, 230; beet pulp 12% sugar, 178; MervoBest formaldehyde-treated rapeseed meal 33% CP, 69; MervoBest formaldehyde-treated soybean meal 45% CP, 68; limestone 36% Ca, 24; monocalcium phosphate, 16; NaCl, 16; urea, 16; magnesium oxide, 16; trace mineral and vitamin premix, 8; Bergafat F100, 8; TiO_2 was included at 0.25% of concentrate DM.

[5]NA = not analyzed.

[6]Intestinal digestible protein (CVB, 2018).

[7]RDP balance (CVB, 2018).

[8]NE_L calculated with the VEM system (CVB, 2018).

cess to drinking water throughout the entire experiment. Cows within each block were adapted to the basal TMR in a freestall barn for 7 d. They were subsequently moved to individual tiestall housing for 13 d of adaptation to the dietary treatments and restriction in movement in preparation for the 4-d measurement period (described below). Each block was housed separately in the freestall barn to facilitate ad libitum feed intake measurement of each block of 4 cows. The average feed intake of the block during the final 3 d of the 7-d freestall ad libitum intake period was used to set a fixed daily feed allocation for individual cows within the block. This fixed amount (equal for all cows within a block) was fed during the 13-d tiestall period and 4-d measurement period. Fresh feed was allocated twice daily at 0500 and 1530 h (half of the daily allowance at each time point). The forage and concentrate portions were deposited into the feed bin in front of the individual cow and mixed by hand into a TMR using a pitchfork according to a standard operating procedure to ensure uniformity. The forage portion (corn silage + grass silage) of the diet was mixed twice weekly using a self-propelled mixer wagon (Strautmann Verti-Mix 500, Bad Laer, Germany) equipped with a cutter loader system and an electronic weighing scale. This mixture was portioned for individual cows and stored at 4°C for no longer than 4 d before feeding. The concentrate was manufactured by Research Diet Services B.V. (Wijk bij Duurstede, the Netherlands) and contained 0.25% titanium dioxide as an inert marker for estimation of apparent total-tract nutrient digestibility (**ATTD**). The concentrate was portioned for individual cows and stored at room temperature before feeding. Feed refusals at each feeding time point were collected and weighed to determine daily feed intake.

Within blocks, cows were randomly assigned to treatments which consisted of the basal TMR (**CON**) or the basal TMR including 1 of 3 iso-MP supplements: (1) 315-g mixture of RP soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily (**ST-RPSR**); (2) 384-g mixture of RP His (hydrogenated fat-coated prototype; Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition), and Met (Smartamine M; Adisseo, France) fed daily (**ST-RPAA**); and (3) 768-g mixture of RP His, Lys, and Met fed every other day (**OS-RPAA**). The basal TMR with the addition of supplements was designed to deliver 100% of required MP over

a 48-h period. The RP soybean meal and rapeseed meal supplement consisted of iso-MP contributions from RP soybean meal (114 g DM/d) and RP rapeseed meal (162 g DM/d). The RP AA mixture of His, Lys, and Met was formulated to deliver digestible AA in amounts relative to their concentration in casein. Based on manufacturer specifications, 103, 252, and 29 g/d of RP His, Lys, and Met (product basis), respectively, was expected to provide 21, 63, and 17 g/d of digestible His, Lys, and Met, respectively (Table 2). Half of the daily dose of RP supplement was delivered with fresh feed in the morning and half was delivered with fresh feed in the afternoon (1530 h) according to the static or oscillating feeding frequency (Figure 1). The RP supplements were mixed into the TMR at the time of feeding as described above.

Table 2. Intestinal digestible protein balance and digestible EAA supply from the rumen-protected protein supplements and TMR

	Supplement[1]		TMR[2]		
Item	RPSR	RPAA	CON	RPSR	RPAA
DVE balance,[3] g/d					
Requirement			1,645	1,645	1,645
Supply			1,564	1,654	1,654
Balance			−81	9	9
Digestible EAA supply,[4] g/d					
His	4	21	45	49	66
Met	2	17	36	38	53
Phe	7	0	107	114	107
Trp	5	0	83	88	83
Ile	6	0	110	116	110
Leu	12	0	184	196	184
Val	7	0	118	125	118
Arg	9	0	118	127	118
Lys	8	63	150	158	213
Thr	6	0	107	113	107
Total	66	101	1,059	1,125	1,160

[1]RPSR = mixture of rumen-protected soybean meal and rumen-protected rapeseed meal with iso-MP contributions from both sources (MervoBest; Agrifirm, the Netherlands); RPAA = mixture of rumen-protected His (Ajinomoto Co., Japan), rumen-protected Lys (AjiPro-L; Ajinomoto Health & Nutrition), and rumen-protected Met (Smartamine M; Adisseo, France) in the profile of casein.

[2]CON = basal TMR with no supplement; RPSR = basal TMR plus RPSR supplement; RPAA = basal TMR plus RPAA supplement.

[3]Intestinal digestible protein (DVE) balance estimated using CVB (2018) based on observed DMI and calculated DVE content of each TMR.

[4]Estimated using NRC (2001) for the RPSR supplement and the CON TMR. Estimated according to product specifications for the RPAA supplement where rumen-protected His, Lys, and Met delivered 20, 25, and 60 g digestible AA/100 g of product, respectively.

Climate Respiration Chamber Housing

After 13 d of adaptation in tie stalls, cows were moved to climate respiration chambers (**CRC**) for a 4-d measurement period to facilitate determination of gaseous exchange and energy and N balance (Figure 1). Measurements and sample collection for determination of energy balance are described below, but were not central to our hypotheses. Therefore, all data on energy balance from this experiment are described in Supplemental Table S1 (see Notes). Detailed descriptions of the CRC design and gas measurements are given by Heetkamp et al. (2015) and van Gastelen et al. (2015). Briefly, individual CRC (11.8 m^2; volume of 34.5 m^3) were designed with thin walls equipped with windows to allow audio and visual contact between cows and minimize the physiological and behavioral effects of social isolation. Inside each chamber the ventilation rate was 43 m^3/h, relative humidity was maintained at 80%, and the temperature was maintained at 10°C. Relative humidity was monitored by a relative humidity sensor in each chamber (Novasina Hygrodat100, Novasina AG, Lachen, Switzerland), and the temperature was monitored by 5 PT100 temperature sensors (Sensor Data BV, Rijswijk, the Netherlands) evenly distributed over the chamber at animal height. Cows were exposed to 16 h of light per day (0500 to 2100 h).

Gas analysis was performed as described by van Gastelen et al. (2015), where the 4 CRC shared a single gas analyzer (ABB Advance Optima AO2000 systems, ABB, Berlin, Germany) that measured gas from each compartment in 12-min intervals. Calibration gases were sampled once daily instead of inlet air. The analyzed and actual values of these calibration gases were used to correct the analyzed gas concentrations from the inlet and exhaust air of the 4 CRC. Gas concentrations and ventilation rates were corrected for pressure, temperature, and relative humidity to arrive at standard temperature and pressure dew point volumes of inlet and exhaust air. Consumption of O_2 and production of CO_2 and CH_4 inside each chamber was calculated from the difference between inlet and exhaust gas volumes. Gas measurements during time points when staff entered the CRC compartments for milking and feeding (maximum 30 min) were discarded from the data analysis. Consumption of O_2 and production of CO_2 and CH_4 was assumed to be linear between the last data point before opening and the first data point after closing the CRC. At the start and the end of the experiment, CO_2 recovery in the CRC was checked by releasing known amounts of CO_2 into each chamber and comparing the known values with data from the gas analysis system. The recovered amounts of CO_2 were between 99.4% and 100.5% (100.1% ± 0.45%).

Measurements and Sample Collection

Energy and N balance and ATTD were based on manure and fecal collection, O_2 consumption, and CO_2 and CH_4 production during the 4-d measurement period (Figure 1). Cows were weighed once at the end of each measure-

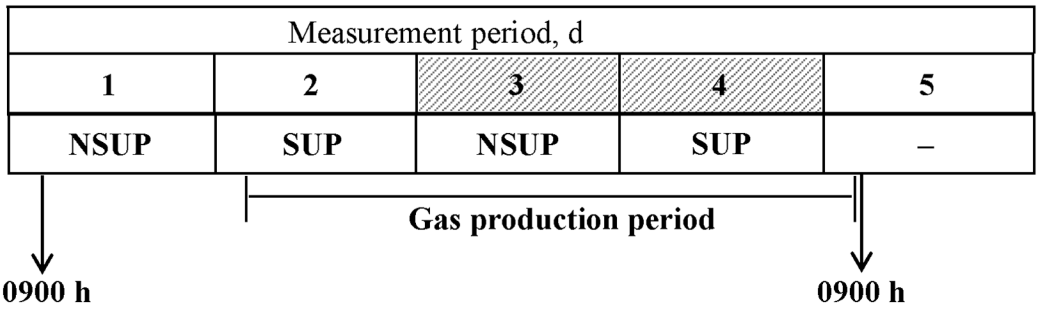

Figure 1. Design of a single 4-d measurement period. Total collection of manure and volatile N began when cows entered the climate respiration chambers at 0900 h on d 1 and ended when cows were removed at 0900 h on d 5. Data considered for measurements of gas production and consumption were from 0800 h on d 2 until 0800 h on d 5. NSUP = days that cows on the oscillating rumen-protected AA treatment did not receive supplement. SUP = days that cows on the oscillating rumen-protected AA treatment did receive supplement. Blood samples were collected on d 3 and 4 (shaded).

ment period immediately before they were removed from the CRC (0900 h). The tiestall platforms in the CRC were equipped with a weighing scale (HBM GmbH, Schüttorf, Germany). The cow-free platform weight (including debris that accumulated during the measurement period) was subtracted from the weight of the platform plus the cow to estimate cow BW. The manure (urine + feces) produced within each CRC compartment during the 4-d period was separately and quantitatively collected, weighed, and mixed. Manure samples of 1 L were collected and stored at −20°C until analysis. Outflowing air from each CRC compartment was directed through a 25% sulfuric acid solution (wt/wt; 125 mL per chamber) to trap aerial ammonia. Samples were collected from this acid trap (125 mL) and from condensed water (approximately 200 mL) from the chamber heat exchanger to quantify contribution of N from volatilized ammonia appearing from excreted and mixed urine and feces. These samples were stored at 4°C until analysis. Feces was collected by rectal grab sampling at 0500 and 1530 h during the measurement period (8 samples per cow) and immediately pooled into a composite sample (100 g fresh feces per sample to yield a composite sample of 800 g) by cow, which was stored at −20°C until analysis. Feed refusals, when present, were collected during the measurement period and stored at 4°C. After each period they were pooled by cow, sampled, and stored at −20°C until analysis.

Cows were milked twice daily at 0500 and 1530 h during the adaptation and measurement periods. Milk weight was recorded at each milking. Milk samples for standard milk composition were collected from individual cows at each milking during the measurement period into tubes containing sodium azide. These were stored at 4°C until analysis within 4 d. An additional milk sample for gross energy (**GE**) and N analyses was collected and pooled by cow at each milking (5 g/kg milk produced at each milking) and stored at −20°C. Samples (approximately 500 g) of corn silage, grass silage, and concentrate were collected twice weekly during feed preparation. These samples (2 samples of 500 g) were pooled per week and stored at −20°C until analysis. Blood samples were collected into 10-mL sodium heparin and potassium EDTA Vacutainers (Becton Dickinson, Rutherford, NJ) by venipuncture of the coccygeal vessels and the subcutaneous abdominal vein of each cow at 0630, 0830, 1030, 1230, and 1430 h on d 3 and 4 of each measurement period. Arteriovenous (**AV**) differences across the tail are assumed to be negligible and thus samples from the coccygeal vessels are representative of mammary arterial supply (Emery et al., 1965). Samples were collected from the left and right subcutaneous abdominal veins, alternating at each time point, to account for differences between sides. Sampling was performed on d 3 and d 4 to capture a nonsupplemented and supplemented day for the OSRPAA treatment (Figure 1). Collection tubes were immediately placed in ice and were centrifuged at 3,000 × *g* for 15 min at 4°C after each sampling time point. Total plasma from each time point was collected and stored in 1-mL polypropylene vials at −20°C until analysis.

Analytical Procedures

Samples of corn silage, grass silage, concentrate, manure, and feces were thawed at room temperature, oven-dried at 60°C until a constant weight was reached, and ground to pass a 1-mm screen using a Wiley mill (Peppink 100AN, Olst, the Netherlands). Wet chemical analysis for DM, ash, NH_3, crude fat, starch, sugars, NDF, ADF, ADL, and titanium was performed as described by Nichols et al. (2018). Fresh samples of silages and feces were used to determine N concentration. Nitrogen in corn silage, grass silage, concentrate, manure, and feces was analyzed by combustion (ISO 16634–1; ISO, 2008). Crude protein content was calculated as total analyzed N × 6.25. An adiabatic bomb calorimeter (IKA-

C700, Janke and Kunkel, Heitersheim, Germany) was used for determination of GE content (ISO 9831; ISO, 1998). Corn silage, grass silage, and concentrate samples were analyzed for DM, ash, N, crude fat, starch (except grass silage), sugars (except corn silage), NDF, ADF, ADL, GE, and titanium (concentrate only). The RPSR mixture was analyzed for DM, ash, N, crude fat, starch, sugars, NDF, ADF, ADL, and GE. The RPAA mixture was analyzed for DM, ash, N, crude fat, and GE. Samples of refused feed were analyzed for DM. Manure samples were analyzed for DM, N, and GE. Fecal samples were analyzed for DM, ash, N, crude fat, starch, NDF, GE, and titanium. Samples of condensed water and the sulfuric acid solution were analyzed for N using the Kjeldahl method with $CuSO_4$ as a catalyst (ISO 5983; ISO, 2005). Reported values for nutrient content of the basal TMR were calculated from ration composition and analyzed values obtained for the forages and concentrate. The NE_L was calculated with the VEM (feed unit lactation) system according to Van Es (1978). Reported intestinal digestible protein (**DVE**), RDP balance (**OEB**), and NE_L were obtained by near-infrared spectroscopy analysis for corn silage and grass silage (Eurofins Agro, Wageningen, the Netherlands). For the concentrate, DVE, OEB, and NE_L were calculated based on table values for composition of the ingredients (CVB, 2018). For the basal TMR, these were calculated from ration composition of all forage and concentrate ingredients. For the supplements, DVE, OEB, and NE_L were derived from manufacturer specifications.

Individual milk samples from each morning and afternoon milking were analyzed for protein, fat, lactose, and urea by mid-infrared spectroscopy (ISO 9622; ISO, 2013; VVB, Doetinchem, the Netherlands). Pooled milk samples were analyzed for GE and N (combustion method) in fresh material as described above. Blood plasma from each time point and day was analyzed for AA. Plasma (200 µL) was mixed (1:0.25) with 10% sulphosalicylic acid containing 2.5 m*M* L-norleucine (product no. N1398, Sigma-Aldrich) as an internal standard and stored at 4°C overnight for deproteinization. Deproteinized plasma was subsequently centrifuged (20,000 × *g* for 10 min at 4°C) and 200 µL of supernatant was mixed with 200 µL of dilution buffer (0.12 *M* Li, pH 2.20; Sykam Chromatography, Eresing, Germany; mixture of supernatant and dilution buffer contained a final concentration of 0.5 m*M* L-norleucine). The mixture was passed through a 0.45-µm nylon syringe filter before analysis. Amino acid analysis was performed using cation-exchange chromatography on a Sykam S433 automated AA analyzer (Sykam Chromatography), using lithium as the counter-ion and postcolumn ninhydrin derivatization (Spackman et al., 1958) and visual detection at 440 and 570 nm. Due to interference from an unknown ninhydrin positive system peak (presumably due to ammonia breakthrough on the ammonia trap) which co-eluted with Lys, cation-exchange chromatography did not produce reliable results for Lys. Therefore, Lys was analyzed using precolumn phenyl isothiocyanate derivatization liquid chromatography. Deproteinized plasma was derivatized with phenyl isothiocyanate according to the protocol of Sherwood (2000) and measured using a Waters Pico-Tag analytical column (3.9 × 150 mm; Waters, Milford, MA) on a Dionex Ultimate 3000 equipped with a quaternary gradient pump, autosampler, and diode array detector (Thermo Fisher, Waltham, MA). Separation was performed as described by Sarwar and Botting (1990) with adjustments to mobile phase A (50 m*M* potassium dihydrogen phosphate with 0.1% triethylamine and 5% acetonitrile adjusted to pH 6.4) and mobile phase B (15 m*M* potassium dihydrogen phosphate with 0.03% triethylamine and 60% acetonitrile adjusted to pH 6.4).

Calculations and Statistical Analysis

Heat production (kJ/d) was calculated as $16.175 \times VO_2$ (L/d) + $5.021 \times VCO_2$ (L/d) where VO_2 and VCO_2 are volumes of O_2 consumed and CO_2 produced, respectively (Gerrits et al., 2015). Apparent total-tract digestibility was calculated considering the nutrient inflow from the basal diet and the treatment supplements. Plasma concentrations of AA were averaged per day over the 5 sampling time points. Milk CP was assumed to consist of 94.5% true protein (DePeters and Ferguson, 1992). All following calculations were based on this estimate of true protein yield. Mammary plasma flow (**MPF**) across the whole udder was estimated according to the Fick principle using Phe and Tyr as internal markers (Cant et al., 1993), where MPF (L/h) = [milk Phe + Tyr output (µmol/h)]/[AV Phe + Tyr difference (µmol/L)], with an allowance for 3.37% contribution of blood-derived proteins to milk Phe + Tyr (Lapierre et al., 2012). Milk output of Phe + Tyr was estimated from the afternoon milk protein yield on the corresponding day of blood sampling (d 3 or d 4), using mean Phe and Tyr concentrations in milk protein reported by Mepham (1987) and Lapierre et al. (2012). Uptakes (mmol/h) of metabolites across the mammary glands were calculated as the product of their plasma AV differences and MPF. Positive uptakes indicate a net removal from plasma, whereas negative values indicate net release from the mammary glands. Mammary clearances were calculated from the model of Hanigan et al. (1998), where clearance (L/h) = (AV difference × MPF)/venous concentration. The average milk protein AA composition reported by Mepham (1987) and Lapierre et al. (2012) and milk protein yield from the afternoon milking on each respective d of blood sampling were used to cal-

culate mammary gland AA uptake to milk true protein output ratios (**U:O**).

Facility limitations impeded implementation of covariate measurements in this study and limited the number of animals available for use. With 7 cows per treatment, we estimated that a 10% difference in yield of total milk and milk protein could be detected based on SEM from similarly designed studies (Warner et al., 2015; Hatew et al., 2015; Klop et al., 2016). For variables such as plasma AA concentrations and mammary AV differences, statistical power was estimated at ≥80% when $\alpha = 0.05$. One cow developed mastitis during the adaptation period and was thus removed from the statistical analysis (n = 6 for OS-RPAA; n = 7 for all other treatments). All data were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). The Kenward-Roger correction was used to adjust the denominator degrees of freedom. Variances in energy and N balance, ATTD, and 4-d means of milk yield, milk composition, and DMI were analyzed using a model assuming the fixed effect of treatment and random effect of block. Variances in milk and component yields, arterial plasma AA concentrations, AV differences, MPF, mammary AA uptakes, clearances, and U:O across nonsupplemented and supplemented days were analyzed using a model assuming the fixed effects of treatment, supplementation phase, and their interaction, and the random effect of block. Multiple comparisons between treatment means were made using the Tukey-Kramer method. When the interaction between treatment and supplementation phase was significant ($P \leq 0.10$), the SLICE option was used to determine the effect of supplementation phase within treatment. Differences between treatments and between supplementation phases within treatment were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Day-by-day treatment means corresponding to the supplementation phase of the OS-RPAA treatment are displayed in Supplemental Tables S2 to S7 (see Notes).

RESULTS AND DISCUSSION

Dry matter intake was not affected by RP protein source or frequency of RP AA supplementation ($P = 0.91$; Table 3). Compared with CON, the additional DMI from the RP supplements over the 4-d measurement period was designed to be 0.34 kg/d on average, aligning well with the realized average increase in DMI of 0.36 kg/d. Apparent total-tract digestibility of crude fat decreased with ST-RPAA and OS-RPAA compared with CON and RPSR ($P < 0.01$; Table 4) due to the rumen-protective technology applied to the AA. Unlike soybean meal and rapeseed meal which were treated with formaldehyde to reduce their ruminal degradation, His, Lys, and Met were coated at least partially in hydrogenated fat (predominantly C18:0) combined with other organic or synthetic polymers to reduce their degradation in the rumen. The coating is designed to release the encapsulated AA upon reaching the abomasum, where the change in pH causes the coating to degrade (RP Met) or the presence of digestive enzymes degrade the coating (RP His and RP Lys; Wu and Papas, 1997). With both technologies, the AA is released into digesta for absorption. The fat-containing coating may not have been completely digested or absorbed, thus resulting in higher fecal crude fat excretion when RP AA were fed. Similarly, Zang et al. (2021) observed reduced ATTD of fat when supplementing a 211 g/d mixture of RP His, RP Lys, and RP Met to dairy cattle diets. Assuming the ATTD of crude fat of 76.0% with CON represents crude fat digestibility in the basal TMR, calculated ATTD of fat in the RP AA supplement was 29.4 and 34.9% for ST-RPAA and OS-RPAA, respectively. This agrees with the intestinal digestibility of 31% for >90% C18:0 sources estimated by Daley et al. (2020). Apparent total-tract CP digestibility did not differ ($P = 0.92$) between treatments, suggesting that net digestion and absorption of nitrogenous components in the supplements was similar along the gastrointestinal tract. Neither RP protein source nor frequency of RP AA supplementation affected ATTD of DM, OM, NDF, starch, or GE ($P > 0.73$).

Static Supplementation of RP Protein Sources

Static daily supplementation of RP soybean meal and rapeseed meal or RP AA provided similar amounts of extra MP but resulted in different estimated digestible AA profiles, where ST-RPAA supplied 35%, 35%, and 39% more His, Lys, and Met per day, respectively, compared with ST-RPSR. The differences in arterial AA concentrations between ST-RPSR and ST-RPAA reflect this difference in digestible AA profile (Table 5). In line with the 36% higher digestible supply of total His, Lys, and Met (**HLM**) with ST-RPAA compared with ST-RPSR, arterial concentration of HLM was 41% higher with ST-RPAA compared with ST-RPSR ($P < 0.01$). Individually, Met concentration was higher ($P < 0.01$) and His and Lys concentration tended to be higher ($P = 0.06$) with ST-RPAA versus ST-RPSR. This agrees with others (Giallongo et al., 2016; Zang et al., 2021; Van den Bossche et al., 2023) who reported increased arterial concentrations of His, Lys, and Met when these AA were supplemented in a RP form to dairy cattle diets. The increase in arterial concentration of His and Met in the current study drove the increase in total group 1 AA concentration (those AA taken up by the mammary gland 1:1 relative to their output in milk protein; Lapierre et al., 2012) with ST-RPAA over ST-RPSR ($P = 0.05$), as there was no difference in the arterial

Table 3. Dry matter intake, milk production, and milk composition of lactating dairy cows receiving rumen-protected protein supplements differing in AA profile and supplementation frequency[1]

	Treatment[2]					
Item	CON	ST-RPSR	ST-RPAA	OS-RPAA	SEM	*P*-value
DMI, kg/d	18.4	18.8	18.8	18.7	0.63	0.91
Yield						
Milk, kg/d	30.1	30.9	29.5	31.7	1.79	0.25
CP, g/d	981	1,006	1,049	1,025	40.1	0.14
Fat, g/d	1,361[ab]	1,299[b]	1,401[ab]	1,489[a]	79.0	0.01
Lactose, g/d	1,407	1,453	1,385	1,449	83.5	0.48
Composition, g/kg						
CP	32.7[b]	32.8[b]	35.9[a]	32.8[b]	1.01	<0.01
Fat	45.7[ab]	42.6[b]	48.1[a]	47.9[a]	1.21	<0.01
Lactose	46.6	47.0	46.9	45.9	0.36	0.18
FPCM,[3] kg/d	31.9[ab]	31.5[b]	32.5[ab]	34.1[a]	1.68	0.05
Milk urea, mg/dL	17.0	15.6	18.4	21.3	1.82	0.17

[a,b]Means within a row with no common superscripts differ ($P < 0.05$).

[1]Data are LSM from the 4-d measurement period.

[2]CON = basal TMR with no supplement; ST-RPSR = basal TMR plus 315-g mixture of rumen-protected (RP) soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily; ST-RPAA = basal TMR plus 384-g mixture of RP His (Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition), and Met (Smartamine M; Adisseo, France) fed daily; OS-RPAA = basal TMR plus 768-g mixture of RP His, Lys, and Met fed every other day. The basal TMR plus supplements were designed to deliver 100% of required MP over a 48-h period. ST-RPSR consisted of iso-MP contributions from RP soybean meal and RP rapeseed meal. ST-RPAA and OS-RPAA delivered digestible AA in amounts relative to their concentration in casein.

[3]Fat- and protein-corrected milk (FPCM; kg/d) = (0.337+ 0.116 × fat % + 0.06 × protein %) × milk yield (kg/d) (CVB, 2018).

concentration of the other group 1 AA (Phe+Tyr and Trp) between ST-RPAA and ST-RPSR ($P \geq 0.29$).

Static supplementation of RP AA decreased the arterial concentration of Leu ($P \leq 0.04$) and tended to decrease the concentration of Val ($P = 0.09$) compared with ST-RPSR and CON, but the concentration of total branched-chain amino acids (**BCAA**) did not differ between CON, ST-RPAA, and ST-RPSR ($P \geq 0.21$). Effects of supplemental His, Lys, and Met on BCAA concentrations are variable. Previous studies supplementing RP His, Lys, and Met together did not report decreases in arterial concentrations of individual BCAA (Giallongo et al., 2016; Zang et al., 2021; Van den Bossche et al., 2023). However, when considering the supplementation of RP His, Lys, or Met individually, Berthiaume et al. (2006) reported that the arterial concentration of Leu and Val tended to decrease

Table 4. Apparent total-tract digestibility (%) of nutrients in lactating dairy cows receiving rumen-protected protein supplements differing in AA profile and supplementation frequency[1]

	Treatment[2]					
Item	CON	ST-RPSR	ST-RPAA	OS-RPAA	SEM	*P*-value
DM	74.5	74.2	74.3	74.5	1.42	0.98
OM	76.1	75.7	75.8	76.2	1.36	0.97
CP	72.3	72.3	72.8	72.0	1.50	0.92
NDF	60.4	60.2	61.3	61.5	2.64	0.93
Crude fat	76.0[a]	75.1[a]	64.9[b]	66.1[b]	1.35	<0.01
Starch	98.0	98.2	98.0	98.0	0.22	0.73
Gross energy	74.4	73.8	73.6	74.5	1.47	0.87

[a,b]Means within a row with no common superscripts differ ($P < 0.05$).

[1]Data are LSM calculated from feed and feces sampled during the 4-d measurement period and were calculated considering the total nutrient inflow from the basal TMR plus supplements.

[2]CON = basal TMR with no supplement; ST-RPSR = basal TMR plus 315-g mixture of rumen-protected (RP) soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily; ST-RPAA = basal TMR plus 384-g mixture of RP His (Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition), and Met (Smartamine M; Adisseo, France) fed daily; OS-RPAA = basal TMR plus 768-g mixture of RP His, Lys, and Met fed every other day. The basal TMR plus supplements were designed to deliver 100% of required MP over a 48-h period. ST-RPSR consisted of iso-MP contributions from RP soybean meal and RP rapeseed meal. ST-RPAA and OS-RPAA delivered digestible AA in amounts relative to their concentration in casein.

Table 5. Arterial plasma concentrations (μ*M*) of AA and AA metabolites in lactating dairy cows receiving rumen-protected protein supplements differing in AA profile and supplementation frequency

	Treatment[1]					
Item	CON	ST-RPSR	ST-RPAA	OS-RPAA	SEM	*P*-value
EAA[2]	791	780	758	764	36.4	0.92
Group 1[3]	174[ab]	160[b]	182[a]	180[ab]	7.1	0.05
Group 2[4]	661	661	612	621	33.1	0.61
BCAA[5]	421	426	360	382	23.3	0.16
HLM[6]	116[b]	113[b]	159[a]	161[a]	7.4	<0.01
NEAA[7]	1,239	1,330	1,321	1,266	37.8	0.28
TAA[8]	2,030	2,111	2,079	2,043	54.0	0.72
Arg	73	72	79	74	4.9	0.79
His	35	30	44	37	4.3	0.09
Ile	125	132	121	133	9.5	0.77
Leu	106[a]	104[a]	81[b]	88[ab]	6.0	0.01
Lys	65[c]	68[bc]	84[ab]	90[a]	4.3	<0.01
Met	17[a]	15[a]	30[b]	34[b]	2.6	<0.01
Phe	42	40	38	38	1.6	0.20
Thr	102	94	89	76	7.8	0.13
Trp	37	35	34	34	2.2	0.69
Val	190	190	158	161	10.6	0.08
Ala	228	225	257	226	13.9	0.22
Asn	40	44	42	38	1.9	0.23
Asp	6.0	7.9	7.4	7.2	0.91	0.56
Cit	80	81	70	69	5.7	0.29
Gln	261[a]	304[ab]	324[b]	326[b]	15.1	0.01
Glu	64	67	68	66	4.2	0.83
Gly	302	339	296	290	20.6	0.18
Orn	40	39	42	39	2.0	0.66
Pro	80	84	88	81	5.5	0.64
Ser	94	100	91	91	4.1	0.40
Tyr	44[a]	40[ab]	35[b]	36[b]	2.0	0.02
1 Methyl-histidine	4.8	4.8	4.9	4.5	0.45	0.93
3 Methyl-histidine	6.3	6.8	7.0	7.0	0.70	0.84

[a-c]Means within a row with no common superscripts differ ($P < 0.05$).

[1]CON = basal TMR with no supplement; ST-RPSR = basal TMR plus 315-g mixture of rumen-protected (RP) soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily; ST-RPAA = basal TMR plus 384-g mixture of RP His (Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition), and Met (Smartamine M; Adisseo, France) fed daily; OS-RPAA = basal TMR plus 768-g mixture of RP His, Lys, and Met fed every other day. The basal TMR plus supplements were designed to deliver 100% of required MP over a 48-h period. ST-RPSR consisted of iso-MP contributions from RP soybean meal and RP rapeseed meal. ST-RPAA and OS-RPAA delivered digestible AA in amounts relative to their concentration in casein.

[2]EAA = Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.

[3]Group 1 = His, Met, Phe+Tyr, Trp.

[4]Group 2 = Arg, Ile, Leu, Lys, Thr, Val.

[5]BCAA = Ile, Leu, Val.

[6]HLM = His, Lys, Met.

[7]NEAA = Ala, Asn, Asp, Cit, Gln, Glu, Gly, Orn, Pro, Ser, Tyr.

[8]TAA = EAA + NEAA.

linearly in response to increased supplementation level of RP Met (0, 36, and 72 g of RP Met/d), and Blum et al. (1999) reported decreased arterial concentrations of Ile and Val in response to RP Met supplementation (50 g of Met/d). Abomasal infusion of Met (up to 40 g/d) resulted in linear decreases in arterial concentrations of Val (Guinard and Rulquin, 1995) and Ile, Leu, and Val (Varvikko et al., 1999). Räisänen et al. (2022) reported that supplemental His decreased arterial concentrations of Ile, Leu, and Val, but only when the diet was deficient in total MP. The decreased circulating concentrations of Leu and Val in response to supplemental His, Met, and Lys could result from mechanisms such as enhanced gastrointestinal or peripheral tissue uptake, but these remain unclear based on the current and previous studies.

Concentration of CP in milk increased with ST-RPAA compared with CON and ST-RPSR ($P < 0.01$; Table 3). This is in line with Giallongo et al. (2016) who observed an increase in milk protein concentration when RP His, Lys, and Met were supplemented to a MP-deficient diet, and Zang et al. (2021) who observed an increase in milk true protein concentration with supplementation of RP

His, Lys, and Met to both low- and high-starch diets. Milk CP yield was not affected by ST-RPSR or ST-RPAA compared with CON ($P \geq 0.18$). Milk fat concentration increased with ST-RPAA over ST-RPSR ($P < 0.01$), but did not differ between ST-RPAA and CON ($P = 0.37$) despite a similar increase in crude fat intake with ST-RPAA over ST-RPSR and CON (156 and 144 g/d, respectively) as a result of the hydrogenated fat coating the AA. There is often no effect of RP AA supplementation on milk fat concentration or yield (e.g., Lee et al., 2012; Morris and Kononoff, 2020; Van den Bossche et al., 2023). Aside from the additional fat supply, the increase in milk fat concentration when RP AA were supplemented may be symptomatic of a relatively greater AA imbalance provoked by the supplementation of only His, Lys, and Met compared with the AA profile in the mixture of RP soybean meal and RP rapeseed meal. In particular, His or Met could have contributed to fatty acid synthesis (Owen et al., 2002) if their supply was in excess of the milk protein synthetic potential allowed by the supply of other nonsupplemented EAA. The high level of RP AA supplementation in the current study may be a factor in the discrepancy in the milk fat concentration response compared with others supplementing similar RP AA at lower levels (e.g., Lee et al., 2012; Morris and Kononoff, 2020; Van den Bossche et al., 2023).

Mammary gland metabolism did not shift dramatically in response to the digestible AA profiles supplemented in this study. The AV difference of HLM tended to be greater with ST-RPAA compared with CON ($P = 0.10$; Table 6), where individually the AV difference of Lys increased with ST-RPAA compared with CON ($P = 0.05$) but there were no differences for Met or His ($P \geq 0.65$). Mammary plasma flow and mammary AA uptake (groups and individuals) did not differ between CON, ST-RPSR, and ST-RPAA ($P > 0.10$; Table 7). Clearance of total HLM decreased with ST-RPAA compared with ST-RPSR ($P = 0.04$; Table 8) suggesting that when considered as a group, mammary affinity for the supplemented AA decreased as their arterial supply increased. When His, Lys, and Met were infused intravenously, mammary clearance of His and Met decreased (Yoder et al., 2020), the proportion of mammary uptake of His, Lys, and Met that was used for milk protein decreased, and their intramammary oxidation increased (Huang et al., 2021). In the current study, individual Met clearance decreased with ST-RPAA compared with ST-RPSR ($P = 0.02$) but there were no differences for His or Lys ($P \geq 0.24$). The AV difference of Phe increased with ST-RPAA compared with CON ($P = 0.03$) but the arterial concentration of Phe was not affected ($P = 0.22$), driving the increase in Phe clearance with ST-RPAA compared with CON ($P = 0.01$). This reflects a relatively greater affinity for Phe where mammary cells reduced Phe efflux in support of milk protein synthesis when it was stimulated by His, Lys, and Met supplementation, which was similarly observed by Yoder et al. (2020) during intravenous infusion of His, Lys, and Met. This response is consistent with the approximately 1:1 relationship between mammary uptake to output for Phe as a group 1 AA (Table 9), where the tendency for greater U:O of Phe was reflected in U:O smaller than 1 for Tyr. Clearance of Leu tended to increase with ST-RPAA compared with CON ($P = 0.06$) and ST-RPSR ($P = 0.09$). As a group 2 AA, Leu and the other BCAA are usually taken up by the udder in excess of their output in milk protein where their excess contributes C and N toward NEAA synthesis and glycolytic and tricarboxylic acid cycle intermediates (Lapierre et al., 2012). The maintained AV difference of Leu despite its decreased arterial concentration in response to ST-RPAA compared with CON and ST-RPSR resulted in an increased mammary affinity for Leu, reflecting its importance for milk protein synthesis and intramammary metabolism.

The marginal use efficiency of daily-supplemented RP soybean meal and rapeseed meal was 17%, whereas it was 67% for daily-supplemented RP AA. This value describes the efficiency with which each unit of RP protein supplement is used for milk protein synthesis and was calculated based on the incremental milk protein yield achieved by the supplement (g of milk protein yield/g of supplement protein) after correcting for the milk protein yield allowed by the nonsupplemented portion of the TMR. The latter was estimated based on the milk protein efficiency (g of milk protein/g of feed protein) observed on CON. The disparity in efficiency of use between supplemented RP soybean meal and rapeseed meal and supplemented RP AA is due to the fact that plant protein sources contain NEAA, which will not be used as efficiently for milk protein synthesis (Doepel and Lapierre, 2010) and that the EAA profile of these sources differs from casein (Huhtanen and Hristov, 2010). A marginal use efficiency of 67% for the mixture of RP His, Lys, and Met is higher than marginal efficiencies reported for postruminal infusions of a mixture of all 10 EAA in the profile of casein (e.g., 22%–35%; Nichols et al., 2019a). This high efficiency of transfer of extra AA into milk protein reflects the relatively lower total supplemented EAA supply (101 g/d) compared with postruminal infusion studies (e.g., 562 and 844 g/d; Nichols et al., 2016, 2019a,b), but also the importance of His, Lys, and Met at the mammary gland level in terms of stimulating milk protein synthesis. Despite the high marginal efficiency observed for ST-RPAA, there were no significant differences in whole-body N balance between CON, ST-RPSR,

Table 6. Arteriovenous difference (μ*M*) of AA in lactating dairy cows receiving rumen-protected protein supplements differing in AA profile and supplementation frequency

	Treatment[1]					
Item	CON	ST-RPSR	ST-RPAA	OS-RPAA	SEM	*P*-value
EAA[2]	253	266	274	257	13.6	0.65
Group 1[3]	58	59	66	59	3.3	0.24
Group 2[4]	212	224	227	216	11.4	0.69
BCAA[5]	120	126	124	119	6.3	0.84
HLM[6]	58	61	68	67	3.1	0.08
NEAA[7]	203	212	228	208	12.6	0.54
TAA[8]	456	477	502	477	26.2	0.65
Arg	29	30	31	28	1.7	0.66
His	10	11	11	11	0.5	0.40
Ile	33	33	33	33	1.9	0.99
Leu	46	48	50	49	1.9	0.60
Lys	38[b]	41[ab]	46[a]	45[ab]	2.3	0.03
Met	8	10	11	11	0.7	0.61
Phe	17[a]	18[ab]	21[b]	17[a]	0.9	0.02
Thr	24	28	26	23	2.4	0.51
Trp	3.7	3.0	3.5	3.0	0.97	0.93
Val	41	45	42	38	3.3	0.47
Ala	30	33	32	31	3.6	0.95
Asn	10	10	11	11	0.9	0.53
Asp	2.6	2.1	2.8	2.7	0.41	0.55
Cit	2.7	0.8	2.5	1.4	0.97	0.48
Gln	54	59	66	58	4.5	0.14
Glu	31	31	34	32	2.6	0.76
Gly	10	10	13	10	2.3	0.67
Orn	17	16	17	13	0.9	0.06
Pro	11	14	9	15	2.3	0.35
Ser	19	20	20	17	2.3	0.56
Tyr	16	17	19	17	1.1	0.17

[a,b]Means within a row with no common superscripts differ ($P < 0.05$).

[1]CON = basal TMR with no supplement; ST-RPSR = basal TMR plus 315-g mixture of rumen-protected (RP) soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily; ST-RPAA = basal TMR plus 384-g mixture of RP His (Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition), and Met (Smartamine M; Adisseo, France) fed daily; OS-RPAA = basal TMR plus 768-g mixture of RP His, Lys, and Met fed every other day. The basal TMR plus supplements were designed to deliver 100% of required MP over a 48-h period. ST-RPSR consisted of iso-MP contributions from RP soybean meal and RP rapeseed meal. ST-RPAA and OS-RPAA delivered digestible AA in amounts relative to their concentration in casein.

[2]EAA = Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.

[3]Group 1 = His, Met, Phe+Tyr, Trp.

[4]Group 2 = Arg, Ile, Leu, Lys, Thr, Val.

[5]BCAA = Ile, Leu, Val.

[6]HLM = His, Lys, Met.

[7]NEAA = Ala, Asn, Asp, Cit, Gln, Glu, Gly, Orn, Pro, Ser, Tyr.

[8]TAA = EAA + NEAA.

and ST-RPAA (Table 10). This is in agreement with others who found no effects of supplementing RP His, Lys, and Met on N partitioning (Lee et al., 2012; Van den Bossche et al., 2023). The positive body N retention in our study (on average 33 g N/d) is in line with that estimated in other studies using respiration chambers (van Gastelen et al., 2015; Nichols et al., 2019a,c) or tiestall total collection techniques (Spanghero and Kowalski, 2021; Nichols et al., 2023). Measurements of body N balance are prone to measurement error, especially in terms of volatile N losses during collection, processing, and analysis of samples, which may result in the overestimation of body N accretion (reviewed by Hristov et al., 2019).

Frequency of RP AA Supplementation

We hypothesized that although the digestible EAA profile was the same for ST-RPAA and OS-RPAA, the difference in supplementation frequency between these treatments would affect dynamics of AA absorption and postabsorptive metabolism. Assuming a fractional passage rate for RP AA of 6%/h, as reported for concentrate feed (Satter, 1986; Warner et al., 2013), the average retention time of supplemented RP AA in the rumen would be approximately 17 h. Therefore, the RP His, RP Lys, and RP Met fed on the supplemented day of OS-RPAA would have actually contributed significantly to

Table 7. Mammary gland plasma flow and net AA uptakes in lactating dairy cows receiving rumen-protected protein supplements differing in AA profile and supplementation frequency

	Treatment[1]					
Item	CON	ST-RPSR	ST-RPAA	OS-RPAA	SEM	*P*-value
Plasma flow, L/h	704	684	614	806	78.5	0.39
Net mammary uptake, mmol/h						
EAA[2]	172	179	168	196	14.5	0.50
Group 1[3]	39	39	41	43	2.3	0.59
Group 2[4]	144	152	139	165	12.7	0.47
BCAA[5]	82	85	76	92	7.4	0.49
HLM[6]	39	41	42	54	5.8	0.26
NEAA[7]	137	145	139	160	14.5	0.63
TAA[8]	309	324	307	356	28.3	0.55
Arg	20	21	19	22	2.2	0.69
His	7.1	7.4	7.0	8.6	0.98	0.63
Ile	22	22	20	26	2.9	0.51
Leu	32	32	30	39	3.7	0.35
Lys	26	28	29	36	3.6	0.19
Met	6.7	6.7	6.6	9.2	1.35	0.46
Phe	12	12	13	12	0.6	0.36
Thr	16	19	16	15	1.7	0.36
Trp	2.5	2.0	2.1	1.2	0.86	0.71
Val	28	30	25	26	2.1	0.28
Ala	20	23	19	26	3.6	0.59
Asn	6.4	6.9	6.7	9.2	1.10	0.23
Asp	1.7	1.4	1.7	2.2	0.40	0.43
Cit	1.8	0.7	1.5	1.7	0.87	0.77
Gln	38	40	41	48	5.8	0.61
Glu	21	21	21	25	2.5	0.51
Gly	6.4	6.9	8.2	9.5	1.99	0.69
Orn	12	11	10	10	0.7	0.13
Pro	7.4	9.2	5.5	8.8	1.37	0.22
Ser	12	14	12	10	1.7	0.30
Tyr	11	11	12	12	0.5	0.38

[1]CON = basal TMR with no supplement; ST-RPSR = basal TMR plus 315-g mixture of rumen-protected (RP) soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily; ST-RPAA = basal TMR plus 384-g mixture of RP His (Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition), and Met (Smartamine M; Adisseo, France) fed daily; OS-RPAA = basal TMR plus 768-g mixture of RP His, Lys, and Met fed every other day. The basal TMR plus supplements were designed to deliver 100% of required MP over a 48-h period. ST-RPSR consisted of iso-MP contributions from RP soybean meal and RP rapeseed meal. ST-RPAA and OS-RPAA delivered digestible AA in amounts relative to their concentration in casein.

[2]EAA = Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.

[3]Group 1 = His, Met, Phe+Tyr, Trp.

[4]Group 2 = Arg, Ile, Leu, Lys, Thr, Val.

[5]BCAA = Ile, Leu, Val.

[6]HLM = His, Lys, Met.

[7]NEAA = Ala, Asn, Asp, Cit, Gln, Glu, Gly, Orn, Pro, Ser, Tyr.

[8]TAA = EAA + NEAA.

intestinal supply and postabsorptive metabolism on the nonsupplemented day. The delay in digestion and absorption of these AA relative to time of feeding is reflected in the measured arterial concentration of His, Lys, and Met. When considered over the oscillation cycle (mean of the nonsupplemented and supplemented day), the arterial concentration of total HLM and individual His, Lys, and Met did not differ between ST-RPAA and OS-RPAA (Table 5). When considering the arterial concentrations between days, concentrations of total HLM and individual His, Lys, and Met did not differ between days for ST-RPAA ($P \geq 0.27$ for supplementation day within ST-RPAA treatment; Table 11), but were higher on the nonsupplemented day compared with the supplemented day for OS-RPAA ($P < 0.01$ for supplementation day within OS-RPAA treatment). Increased concentration of His, Lys, and Met on the nonsupplemented day compared with the supplemented day is in line with the above estimated time delay between the time of feeding, absorption from the small intestine, and appearance in peripheral circulation of His, Lys, and Met after they were delivered in feed at 0500 and 1530 h on the supplemented day.

Milk and component composition or yield did not differ across supplementation day for ST-RPAA or OS-

Table 8. Mammary gland clearance (L/h) of AA in lactating dairy cows receiving rumen-protected protein supplements differing in AA profile and supplementation frequency

	Treatment[1]					
Item	CON	ST-RPSR	ST-RPAA	OS-RPAA	SEM	*P*-value
EAA[2]	337	357	353	391	32.8	0.71
Group 1[3]	354	401	364	360	33.2	0.67
Group 2[4]	342	357	369	420	36.3	0.48
BCAA[5]	289	292	329	367	32.7	0.31
HLM[6]	742[ab]	871[a]	483[b]	608[ab]	96.4	0.05
NEAA[7]	135	130	127	155	14.7	0.53
TAA[8]	200	199	194	228	17.4	0.51
Arg	539	534	419	510	79.0	0.68
His	467	601	258	365	125.7	0.28
Ile	261	234	238	282	36.9	0.78
Leu	581[a]	627[a]	1,111[ab]	1,244[b]	139.5	<0.01
Lys	1,021	1,044	786	862	107.4	0.28
Met	1,175[ab]	1,591[a]	353[b]	550[b]	226.7	0.01
Phe	508[a]	577[a]	804[b]	623[ab]	56.7	0.01
Thr	239	437	277	376	115.4	0.41
Trp	85	80	74	47	32.9	0.82
Val	198	223	221	230	25.7	0.84
Ala	113	128	87	132	20.6	0.41
Asn	215	212	221	346	39.9	0.07
Asp	494	275	474	577	115.4	0.30
Cit	23	5	23	28	12.3	0.58
Gln	185	166	160	180	24.1	0.85
Glu	666	605	666	762	96.4	0.60
Gly	22	21	29	36	7.0	0.42
Orn	523	483	417	384	50.0	0.20
Pro	112	144	74	269	82.9	0.39
Ser	174	181	178	152	28.3	0.76
Tyr	448[a]	539[ab]	852[b]	724[ab]	112.9	0.04

[a,b]Means within a row with no common superscripts differ ($P < 0.05$).

[1]CON = basal TMR with no supplement; ST-RPSR = basal TMR plus 315-g mixture of rumen-protected (RP) soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily; ST-RPAA = basal TMR plus 384-g mixture of RP His (Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition), and Met (Smartamine M; Adisseo, France) fed daily; OS-RPAA = basal TMR plus 768-g mixture of RP His, Lys, and Met fed every other day. The basal TMR plus supplements were designed to deliver 100% of required MP over a 48-h period. ST-RPSR consisted of iso-MP contributions from RP soybean meal and RP rapeseed meal. ST-RPAA and OS-RPAA delivered digestible AA in amounts relative to their concentration in casein.

[2]EAA = Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.

[3]Group 1 = His, Met, Phe+Tyr, Trp.

[4]Group 2 = Arg, Ile, Leu, Lys, Thr, Val.

[5]BCAA = Ile, Leu, Val.

[6]HLM = His, Lys, Met.

[7]NEAA = Ala, Asn, Asp, Cit, Gln, Glu, Gly, Orn, Pro, Ser, Tyr.

[8]TAA = EAA + NEAA.

RPAA ($P \geq 0.15$; Supplemental Table S2). In contrast, Tebbe and Weiss (2020) found higher milk and protein yield on the low-CP day of their 24-h oscillation. The difference between their finding and that of the current study is likely due to the relatively greater magnitude of CP oscillation in their study (11.9% to 16.2% CP, DM basis) compared with the current study (15.9% to 17.1% CP, DM basis). When considered over the oscillation cycle, milk CP concentration was lower with OS-RPAA compared with ST-RPAA ($P = 0.01$; Table 3). This agrees with Tebbe and Weiss (2020) who found lower milk protein concentration over the oscillating cycle in their 24-h oscillating treatment compared with their static 14.1% CP diet. It seems that over time, a consistent daily protein supplementation better supports milk protein synthesis compared with a higher supply provided every 24 h. However, Rauch et al. (2021) and Erickson et al. (2023) reported no differences in milk protein concentration with 48-h oscillation cycles. Differences in milk protein synthesis between 24- and 48-h oscillation periods may arise due to the effect of time period on passage rate of microbial protein derived from urea recycling or mobilization of labile protein stores during the low-CP phase. Further, these previous CP oscillation studies with dairy cattle (Tebbe and Weiss, 2020; Rauch et al., 2021; Erickson et al., 2023) imposed CP oscillations by altering the

ingredient composition of the concentrate portion of the diets, and did not involve supplementation of RP AA as did the present study.

When considered over the oscillation cycle, mammary gland metabolism did not shift dramatically in response to frequency of RP AA supplementation, with no differences observed between ST-RPAA and OS-RPAA for AV difference (except Phe which was lower with OS-RPAA; $P = 0.03$; Table 6), mammary uptake, clearance, or U:O. However, mammary gland metabolism did respond to oscillating supplementation of RP His, RP Lys, and RP Met over the supplementation days. Driven by the individual AV differences of Lys and Met, but not His, the AV difference of total HLM was greater on the nonsupplemented day compared with the supplemented day with OS-RPAA ($P < 0.01$; Table 11). The AV difference of Arg was also greater on the nonsupplemented day compared with the supplemented day with OS-RPAA ($P = 0.04$). Despite no significant treatment × supplementation day interaction, there was a noteworthy 41% numerical increase in MPF on the nonsupplemented day compared with the supplemented day with OS-RPAA (Supplemental Table S5).

Table 9. Mammary gland AA uptake to milk output ratios in lactating dairy cows receiving rumen-protected protein supplements differing in AA profile and supplementation frequency

	Treatment[1]					
Item	CON	ST-RPSR	ST-RPAA	OS-RPAA	SEM	*P*-value
EAA[2]	1.22	1.23	1.10*	1.34	0.082	0.22
Group 1[3]	0.97*	0.95*	0.94*	1.03*	0.038	0.33
Group 2[4]	1.28	1.30	1.14*	1.41	0.091	0.20
BCAA[5]	1.21	1.21	1.04*	1.31	0.092	0.21
HLM[6]	1.09*	1.11*	1.07*	1.44	0.142	0.20
NEAA[7]	0.72	0.74	0.68	0.81	0.064	0.46
TAA[8]	0.93*	0.95*	0.86†	1.04*	0.070	0.30
TAA-N[9]	0.97*	0.98*	0.88*	1.07*	0.071	0.28
Arg	2.57	2.57	2.23	2.79	0.242	0.45
His	1.04*	1.05*	0.95*	1.21*	0.129	0.49
Ile	1.31†	1.26*	1.09*	1.48	0.155	0.35
Leu	1.11*	1.10*	0.99*	1.33	0.117	0.23
Lys	1.18*	1.21*	1.18*	1.59	0.143	0.15
Met	0.94*	0.91*	0.86*	1.23*	0.172	0.40
Phe	1.05†	1.05†	1.05†	1.03*	0.023	0.84
Thr	1.06*	1.15*	0.96*	0.92*	0.088	0.27
Trp	0.84*	0.61*	0.66*	0.36	0.262	0.62
Val	1.28[ab]	1.34[a]	1.08[b]*	1.17[ab]	0.061	0.03
Ala	1.39*	1.50	1.24*	1.68	0.227	0.55
Asn	0.53	0.55	0.51	0.73	0.085	0.19
Asp	0.11	0.08	0.09	0.13	0.022	0.36
Gln	1.49	1.55	1.51	1.84	0.194	0.51
Glu	0.49	0.47	0.44	0.57	0.060	0.42
Gly	0.66†	0.69*	0.79*	0.94*	0.190	0.71
Pro	0.22	0.26	0.15	0.24	0.035	0.14
Ser	0.55	0.59	0.50	0.43	0.070	0.25
Tyr	0.96†	0.96†	0.95	0.97*	0.023	0.84

[a,b]Means within a row with no common superscripts differ ($P < 0.05$).

[1]CON = basal TMR with no supplement; ST-RPSR = basal TMR plus 315-g mixture of rumen-protected (RP) soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily; ST-RPAA = basal TMR plus 384-g mixture of RP His (Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition, USA), and Met (Smartamine M; Adisseo, France) fed daily; OS-RPAA = basal TMR plus 768-g mixture of RP His, Lys, and Met fed every other day. The basal TMR plus supplements were designed to deliver 100% of required MP over a 48-h period. ST-RPSR consisted of iso-MP contributions from RP soybean meal and RP rapeseed meal. ST-RPAA and OS-RPAA delivered digestible AA in amounts relative to their concentration in casein.

[2]EAA = Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.

[3]Group 1 = His, Met, Phe+Tyr, Trp.

[4]Group 2 = Arg, Ile, Leu, Lys, Thr, Val.

[5]BCAA = Ile, Leu, Val.

[6]HLM = His, Lys, Met.

[7]NEAA = Ala, Asn, Asp, Cit, Gln, Glu, Gly, Pro, Ser, Tyr.

[8]TAA = EAA + NEAA.

[9]TAA-N = total AA on an N basis.

*Value does not differ from 1.00 ($P > 0.10$).

†Value does not differ from 1.00 (tendency; $0.10 \geq P > 0.05$); if no symbol, the value differs from 1.00 ($P < 0.05$).

Consistent with the higher arterial concentration of His, Lys, and Met on the nonsupplemented day, the numerically greater MPF could align with previous observations where MPF increased during imbalanced EAA supply in an attempt to maintain extracellular and intracellular concentrations of precursor substrates required for milk component synthesis (Doepel et al., 2016; Yoder et al., 2020; Nichols et al., 2022). However, as this was a numerical and nonsignificant response, the results in the current study should be interpreted with caution as the metabolic adaptation at the mammary gland level that may be occurring during the nonsteady state condition inherent to OS-RPAA is not well understood, and may violate some assumptions of the Fick principle associated with intramammary metabolism of Phe and Tyr or mammary gland use of peptides. Mammary uptake of HLM tended to be greater on the nonsupplemented day compared with the supplemented day ($P = 0.09$; Table 11). Clearance of total HLM and individual His, Lys, and Met did not differ between ST-RPAA and OS-RPAA and were not affected by an interaction between treatment and supplementation day. Given the tendency for increased mammary uptake of HLM but no difference in milk protein output between the nonsupplemented and the supplemented days (Supplemental Table S2), the mammary U:O of total HLM tended to be greater on the nonsupplemented day compared with the supplemented day ($P = 0.06$; Table 11). Individually, the U:O of Lys was greater ($P < 0.01$) on the nonsupplemented day compared with the supplemented day, suggesting greater intramammary AA metabolism on the nonsupplemented day, particularly of Lys. Of the 3 supplemented EAA, Lys is typically sequestered by mammary cells in excess relative to its output in milk and contributes N and C skeletons for intramammary metabolism (group 2 AA; Lapierre et al., 2009, 2012). It therefore follows that the U:O of Lys would display relatively greater flexibility for intramammary metabolism under conditions of oscillating supply compared with His and Met which generally maintain unity with mammary uptake relative to milk protein output.

When supplied every other day over the 4-d measurement period, the marginal use efficiency of OS-RPAA was 49%, in contrast to the 67% estimated for ST-RPAA, indicating that supplementation frequency can alter AA use efficiency. Despite the lower marginal efficiency and milk protein concentration, there were no differences in N partitioning between ST-RPAA and OS-RPAA (Table 10). In contrast to our results, Rauch et al. (2023) reported increased urinary N excretion with a 48-h oscillating CP diet compared with a static CP diet. The increased urinary N excretion on the oscillating diet in Rauch et al. (2023) coincided with a greater CP digestibility compared with the static diet. Dietary CP oscillation reported by Tebbe and Weiss (2020) also resulted in increased ATTD of CP. In our study, oscillating supplementation of 3 RP AA

Table 10. Nitrogen balance of lactating dairy cows receiving rumen-protected protein supplements differing in AA profile and supplementation frequency

	Treatment[1]					
Item	CON	ST-RPSR	ST-RPAA	OS-RPAA	SEM	*P*-value
Nitrogen balance, g/d						
N intake[2]	475	497	504	498	15.8	0.46
N manure	282	290	300	298	7.3	0.26
Fecal N[3]	132	138	138	143	10.8	0.83
Urine N[4]	150	152	163	156	9.6	0.62
N milk	154	158	166	163	6.1	0.12
N condense + acid[5]	8	8	8	9	0.9	0.96
N retention[6]	31	42	30	29	9.8	0.79
Fecal N/N intake	0.28	0.28	0.27	0.28	0.015	0.92
Urine N/N intake	0.32	0.31	0.33	0.32	0.025	0.92
Milk N/N intake	0.32	0.32	0.33	0.33	0.010	0.85

[1]CON = basal TMR with no supplement; ST-RPSR = basal TMR plus 315-g mixture of rumen-protected (RP) soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily; ST-RPAA = basal TMR plus 384-g mixture of RP His (Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition), and Met (Smartamine M; Adisseo, France) fed daily; OS-RPAA = basal TMR plus 768-g mixture of RP His, Lys, and Met fed every other day. The basal TMR plus supplements were designed to deliver 100% of required MP over a 48-h period. ST-RPSR consisted of iso-MP contributions from RP soybean meal and RP rapeseed meal. ST-RPAA and OS-RPAA delivered digestible AA in amounts relative to their concentration in casein.

[2]Basal diet plus supplements.

[3]Fecal N = N intake × [1 − (CP digestibility/100)].

[4]Urine N = N manure − fecal N.

[5]N from condense collected from heat exchanger + N trapped from outflowing air.

[6]N retention = N intake (including infusate N) − N manure − N milk − (N condense + acid).

Table 11. Day-to-day variation in plasma AA concentrations and mammary metabolism in lactating dairy cows receiving rumen-protected protein supplements differing in AA profile and supplementation frequency

	Treatment[1]									*P*-value[2]					
	CON		ST-RPSR		ST-RPAA		OS-RPAA					Supplementation day within treatment			
Item	NSUP	SUP	NSUP	SUP	NSUP	SUP	NSUP	SUP	SEM	Supp	Trt × Supp	CON	ST-RPSR	ST-RPAA	OS-RPAA
Milk CP, %	3.30	3.24	3.28	3.27	3.59	3.60	3.27	3.28	0.010	0.06	<0.01	<0.01	0.67	0.47	0.64
Arterial plasma, μ*M*															
Group 1[3]	178	171	164	156	186	177	193	166	7.6	<0.01	0.05	0.19	0.15	0.11	<0.01
HLM[4]	123	110	118	108	162	155	193	130	8.3	<0.01	<0.01	0.10	0.22	0.38	<0.01
His	36	34	31	29	45	43	45	29	4.4	<0.01	<0.01	0.29	0.51	0.27	<0.01
Leu	111	101	105	103	81	80	83	92	6.5	0.67	0.09	0.06	0.73	0.74	0.09
Lys	69	61	71	65	86	83	105	75	5.0	<0.01	<0.01	0.09	0.24	0.62	<0.01
Met	18	15	16	14	32	29	42	26	2.9	<0.01	<0.01	0.31	0.32	0.38	<0.01
Glu	65	62	64	70	71	65	66	66	4.5	0.61	0.10	0.30	0.10	0.10	0.97
Pro	88	71	87	82	89	88	85	77	5.8	<0.01	0.04	<0.01	0.16	0.82	0.07
Tyr	45	44	41	39	37	33	35	38	2.2	0.39	0.10	0.60	0.39	0.06	0.12
Arteriovenous difference, μ*M*															
HLM	63	54	60	62	70	66	75	59	3.5	<0.01	<0.01	0.01	0.57	0.25	<0.01
Arg	31	27	29	31	32	29	30	26	2.0	0.02	0.09	0.03	0.32	0.14	0.04
His	11	10	10	12	12	11	11	10	0.6	0.56	0.02	0.27	0.01	0.07	0.43
Lys	41	35	41	40	48	45	52	39	2.6	<0.01	0.01	0.01	0.74	0.23	<0.01
Met	11	9	9	11	11	11	12	9	0.9	0.33	0.01	0.05	0.04	0.55	0.01
Glu	31	30	29	34	36	32	32	32	2.9	0.79	0.07	0.56	0.04	0.10	0.74
Pro	17	5	15	13	9	9	17	12	2.8	0.01	0.09	<0.01	0.53	0.91	0.12
Net uptake, mmol/h															
HLM	42	37	44	39	42	42	69	39	8.0	0.08	0.10	0.65	0.69	0.99	0.09
Pro	11.2	3.5	10.3	8.1	5.1	5.8	9.8	7.7	1.68	0.01	0.05	<0.01	0.28	0.74	0.34
Clearance, L/h															
Leu	575	586	596	658	1,246	977	1,556	932	165.4	0.03	0.04	0.95	0.73	0.14	<0.01
U:O[5]															
HLM	1.14*	1.04*	1.15*	1.07*	1.06*	1.09*	1.86	1.02*	0.201	0.09	0.06	0.72	0.77	0.93	0.06
Lys	1.23*	1.13*	1.28*	1.14*	1.18*	1.19*	2.06	1.11*	0.203	0.05	0.10	0.73	0.63	0.96	<0.01
Pro	0.32	0.11	0.29	0.23	0.14	0.16	0.27	0.23	0.046	0.02	0.06	<0.01	0.36	0.72	0.35

[1]CON = basal TMR with no supplement; ST-RPSR = basal TMR plus 315-g mixture of rumen-protected (RP) soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily; ST-RPAA = basal TMR plus 384-g mixture of RP His (Ajinomoto Co., Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition), and Met (Smartamine M; Adisseo, France) fed daily; OS-RPAA = basal TMR plus 768-g mixture of RP His, Lys, and Met fed every other day. The basal TMR plus supplements were designed to deliver 100% of required MP over a 48-h period. ST-RPSR consisted of iso-MP contributions from RP soybean meal and RP rapeseed meal. ST-RPAA and OS-RPAA delivered digestible AA in amounts relative to their concentration in casein. Within treatment, the supplementation phase indicates whether cows on OS-RPAA were receiving the RP supplement (SUP) or not (NSUP).

[2]Supp = effect of supplementation phase. *P*-value for treatment can be found in respective tables. Data were included if the interaction between treatment and supplementation phase (Trt × Supp) was significant ($P < 0.10$). The SLICE option (SAS version 9.4, SAS Institute Inc., Cary, NC) was used to evaluate differences between supplementation phase within a treatment (CON, ST-RPSR, ST-RPAA, or OS-RPAA).

[3]Group 1 = His, Met, Phe+Tyr, Trp.

[4]HLM = His, Lys, Met.

[5]U:O = mammary gland AA uptake to milk true protein output ratios.

*Within U:O, value does not differ from 1.00 ($P > 0.10$); if no symbol, the value differs from 1.00 ($P < 0.05$).

resulted in no difference in CP digestibility or urinary N excretion compared with their static supplementation. This may indicate that the source of protein used to create dietary CP oscillation affects protein digestibility and subsequent postabsorptive AA metabolism.

We did observe some minor day-to-day variation, particularly in arterial AA concentration (Leu, Lys, and Pro) and mammary AV difference (Arg, His, Lys, Met, Glu) for CON, ST-RPSR, and ST-RPAA where technically their nutrient supply was static across the supplemented and nonsupplemented days relative to OS-RPAA (Table 11). There were more differences between days for CON compared with ST-RPSR and ST-RPAA. We speculate that this reflects relatively greater compensatory shifts in nutrient absorption and postabsorptive metabolism occurring at the lower MP supply, and that these shifts are dynamic over time. Others have reported shifts in diurnal patterns of arterial metabolite concentrations in response to varying patterns of feeding or protein supplementation over time (Niu et al., 2014; Rottman et al., 2014; Salfer and Harvatine, 2020). To our knowledge, this is the first characterization of mammary AA metabolism across successive days. The dynamics of mammary gland metabolism as it relates to day-to-day or within-day nutrient supply should be further characterized.

CONCLUSIONS

Compared with a daily-supplemented mixture of RP soybean meal and RP rapeseed meal, a daily-supplemented mixture of RP His, RP Lys, and RP Met in a casein profile increased milk protein concentration and was used with a higher marginal efficiency. Mammary affinity (i.e., clearance) of His, Lys, and Met (when considered as a group) decreased and the affinity of Phe and Leu increased with daily supplementation of RP His, RP Lys, and RP Met compared with RP soybean meal and RP rapeseed meal. The AA profile of RP protein supplements did not affect whole-body N balance. When compared with static daily supplementation, 24-h oscillating supply of RP His, RP Lys, and RP Met decreased milk protein concentration. When RP AA were oscillated, arterial concentrations of His, Lys, and Met increased on the nonsupplemented day compared with the supplemented day. Mammary gland metabolism responded on the nonsupplemented day with a tendency for increased uptake and U:O of His, Lys, and Met as a group and increased U:O of Lys compared with the supplemented day. Frequency of supplementation of RP His, RP Lys, and RP Met did not affect whole-body N balance. Supplemented RP AA were used less efficiently for milk protein production when supplied according to a 24-h oscillating scheme compared with static daily supplementation.

NOTES

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Nonstandard abbreviations used: ATTD = apparent total-tract nutrient digestibility; AV = arteriovenous; BCAA = branched-chain amino acid; CON = basal TMR; CRC = climate respiration chamber; DVE = intestinal digestible protein; FPCM = fat- and protein-corrected milk; GE = gross energy; HLM = total His, Lys, and Met; MPF = mammary plasma flow; NSUP = nonsupplemented day for OS-RPAA treatment; OEB = RDP balance; OS-RPAA = 768-g mixture of RP His, RP Lys, and RP Met fed every other day; RP = rumen-protected; RPAA = mixture of RP His, RP Lys, and RP Met in the profile of casein; RPSR = mixture of RP soybean meal and RP rapeseed meal with iso-MP contributions from both sources; ST-RPAA = 384-g mixture of RP His, Lys, and Met fed daily; ST-RPSR = 315-g mixture of RP soybean meal and RP rapeseed meal fed daily; SUP = supplemented day for OS-RPAA treatment; Supp = effect of supplementation phase; TAA = total AA; Trt = treatment; U:O = mammary gland AA uptake to milk true protein output ratio.

REFERENCES

Archibeque, S. L., H. C. Freetly, N. A. Cole, and C. L. Ferrell. 2007. The influence of oscillating dietary protein concentrations on finishing cattle. II. Nutrient retention and ammonia emissions. J. Anim. Sci. 85:1496–1503. https://doi.org/10.2527/jas.2006-208.

Bach, A., G. B. Huntington, S. Calsamiglia, and M. D. Stern. 2000. Nitrogen metabolism of early lactation cows fed diets with two different levels of protein and different amino acid profiles. J. Dairy Sci. 83:2585–2595. https://doi.org/10.3168/jds.S0022-0302(00)75152-6.

Berthiaume, R., M. C. Thivierge, R. A. Patton, P. Dubreuil, M. Stevenson, B. W. McBride, and H. Lapierre. 2006. Effect of ruminally

protected methionine on splanchnic metabolism of amino acids in lactating dairy cows. J. Dairy Sci. 89:1621–1634. https://doi.org/10.3168/jds.S0022-0302(06)72229-9.

Blum, J. W., R. M. Bruckmaier, and F. Jans. 1999. Rumen-protected methionine fed to dairy cows: Bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. J. Dairy Sci. 82:1991–1998. https://doi.org/10.3168/jds.S0022-0302(99)75435-4.

Cabrita, A. R. J., R. J. Dewhurst, D. S. P. Melo, J. M. Moorby, and A. J. M. Fonseca. 2011. Effects of dietary protein concentration and balance of absorbable amino acids on productive responses of dairy cows fed corn silage-based diets. J. Dairy Sci. 94:4647–4656. https://doi.org/10.3168/jds.2010-4097.

Cant, J. P., E. J. DePeters, and R. L. Baldwin. 1993. Mammary amino acid utilization in dairy cows fed fat and its relationship to milk protein depression. J. Dairy Sci. 76:762–774. https://doi.org/10.3168/jds.S0022-0302(93)77400-7.

Cole, N. A., L. W. Greene, F. T. McCollum, T. Montgomery, and K. McBride. 2003. Influence of oscillating dietary crude protein concentration on performance, acid-base balance, and nitrogen excretion of steers. J. Anim. Sci. 81:2660–2668. https://doi.org/10.2527/2003.81112660x.

CVB (Centraal Veevoederbureau). 2018. CVB Table Ruminants 2018. CVB.

Daley, V. L., L. E. Armentano, P. J. Kononoff, and M. D. Hanigan. 2020. Modeling fatty acids for dairy cattle: Models to predict total fatty acid concentration and fatty acid digestion of feedstuffs. J. Dairy Sci. 103:6982–6999. https://doi.org/10.3168/jds.2019-17407.

DePeters, E. J., and J. D. Ferguson. 1992. Nonprotein nitrogen distribution in the milk of cows. J. Dairy Sci. 75:3192–3209. https://doi.org/10.3168/jds.S0022-0302(92)78085-0.

Doepel, L., I. I. Hewage, and H. Lapierre. 2016. Milk protein yield and mammary metabolism are affected by phenylalanine deficiency but not by threonine or tryptophan deficiency. J. Dairy Sci. 99:3144–3156. https://doi.org/10.3168/jds.2015-10320.

Doepel, L., and H. Lapierre. 2010. Changes in production and mammary metabolism of dairy cows in response to essential and nonessential amino acid infusions. J. Dairy Sci. 93:3264–3274. https://doi.org/10.3168/jds.2009-3033.

Doranalli, K., G. B. Penner, and T. Mutsvangwa. 2011. Feeding oscillating dietary crude protein concentrations increases nitrogen utilization in growing lambs and this response is partly attributable to increased urea transfer to the rumen. J. Nutr. 141:560–567. https://doi.org/10.3945/jn.110.133876.

Emery, R. S., L. D. Brown, and J. W. Bell. 1965. Correlation of milk fat with dietary and metabolic factors in cows fed restricted roughage rations supplemented with magnesium oxide or sodium bicarbonate. J. Dairy Sci. 48:1647–1651. https://doi.org/10.3168/jds.S0022-0302(65)88543-5.

Erickson, M. G., G. I. Zanton, and M. A. Wattiaux. 2023. Dynamic lactation responses to dietary crude protein oscillations in diets adequate and deficient in metabolizable protein in Holstein cows. J. Dairy Sci. 106:8774–8786. https://doi.org/10.3168/jds.2023-23603.

Gerrits, W. J. J., J. J. G. C. van den Borne, and E. Labussière. 2015. Deriving heat production from gaseous exchange: validity of the approach. Pages 19–34 in Indirect Calorimetry. Techniques, Computations and Applications. W. J. J. Gerrits and E. Labussière, ed. Wageningen Academic Publishers, Wageningen, the Netherlands. https://doi.org/10.3920/978-90-8686-809-4.

Giallongo, F., M. T. Harper, J. Oh, J. C. Lopes, H. Lapierre, R. A. Patton, C. Parys, I. Shinzato, and A. N. 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.

Guinard, J., and H. Rulquin. 1995. Effects of graded amounts of duodenal infusions of methionine on the mammary uptake of major milk precursors in dairy cows. J. Dairy Sci. 78:2196–2207. https://doi.org/10.3168/jds.S0022-0302(95)76847-3.

Hanigan, M. D., J. France, D. Wray-Cahen, D. E. Beever, G. E. Lobley, L. Reutzel, and N. E. Smith. 1998. Alternative models for analyses of liver and mammary transorgan metabolite extraction data. Br. J. Nutr. 79:63–78. https://doi.org/10.1079/BJN19980010.

Haque, M. N., H. Rulquin, A. Andrade, P. Faverdin, J. L. Peyraud, and S. Lemosquet. 2012. Milk protein synthesis in response to the provision of an "ideal" amino acid profile at 2 levels of metabolizable protein supply in dairy cows. J. Dairy Sci. 95:5876–5887. https://doi.org/10.3168/jds.2011-5230.

Hatew, B., S. C. Podesta, H. van Laar, W. F. Pellikaan, J. L. Ellis, J. Dijkstra, and A. Bannink. 2015. Effects of dietary starch content and rate of fermentation on methane production in lactating dairy cows. J. Dairy Sci. 98:486–499. https://doi.org/10.3168/jds.2014-8427.

Heetkamp, M. J. W., S. J. J. Alferink, T. Zandstra, P. Hendriks, H. van den Brand, and W. J. J. Gerrits. 2015. Design of climate respiration chambers, adjustable to the metabolic mass of subjects. Pages 35–56 in Indirect Calorimetry. Techniques, Computations and Applications. W. J. J. Gerrits and E. Labussière, ed. Wageningen Academic Publishers, Wageningen, the Netherlands. https://doi.org/10.3920/978-90-8686-809-4.

Hristov, A. N., A. Bannink, L. A. Crompton, P. Huhtanen, M. Kreuzer, M. McGee, P. Nozière, C. K. Reynolds, A. R. Bayat, D. R. Yáñez-Ruiz, J. Dijkstra, E. Kebreab, A. Schwarm, K. J. Shingfield, and Z. Yu. 2019. Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. J. Dairy Sci. 102:5811–5852. https://doi.org/10.3168/jds.2018-15829.

Huang, X., P. S. Yoder, I. A. M. A. Teixeira, and M. D. Hanigan. 2021. Assessing amino acid uptake and metabolism in mammary glands of lactating dairy cows intravenously infused with methionine, lysine, and histidine or with leucine and isoleucine. J. Dairy Sci. 104:3032–3051. https://doi.org/10.3168/jds.2020-18169.

Huhtanen, P., and A. N. Hristov. 2010. Effects of energy and protein supply on milk protein yield responses in dairy cows. Pages 287–298 in Energy and Protein Metabolism and Nutrition. G. M. Crovetto, ed. EAAP publication No 127. Wageningen Academic Publishers, Wageningen, the Netherlands. https://doi.org/10.3920/978-90-8686-709-7.

ISO (International Standards Organization). 1998. Animal feeding stuffs, animal products, and feces or urine. Determination of gross calorific value—Bomb calorimeter method. International Standards Organization, Geneva, Switzerland.

ISO (International Standards Organization). 2005. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content—Part 1: Kjeldahl method. International Standards Organization, Geneva, Switzerland.

ISO (International Standards Organization). 2008. Food products. Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content—Part 1: Oilseeds and animal feeding stuffs. International Standards Organization, Geneva, Switzerland.

ISO (International Standards Organization). 2013. Milk and liquid milk products. Guidelines for the application of mid-infrared spectrometry. International Standards Organization, Geneva, Switzerland.

Kiran, D., and T. Mutsvangwa. 2009. Nitrogen utilization in growing lambs fed oscillating dietary protein concentrations. Anim. Feed Sci. Technol. 152:33–41. https://doi.org/10.1016/j.anifeedsci.2009.03.009.

Klop, G., B. Hatew, A. Bannink, and J. Dijkstra. 2016. Feeding nitrate and docosahexaenoic acid affects enteric methane production and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 99:1161–1172. https://doi.org/10.3168/jds.2015-10214.

Lapierre, H., L. Doepel, E. Milne, and G. E. Lobley. 2009. Responses in mammary and splanchnic metabolism to altered lysine supply in dairy cows. Animal 3:360–371. https://doi.org/10.1017/S1751731108003571.

Lapierre, H., G. E. Lobley, L. Doepel, G. Raggio, H. Rulquin, and S. Lemosquet. 2012. Triennial Lactation Symposium: Mammary metabolism of amino acids in dairy cows. J. Anim. Sci. 90:1708–1721. https://doi.org/10.2527/jas.2011-4645.

Lapierre, H., G. E. Lobley, and D. R. Ouellet. 2021. Histidine optimal supply in dairy cows through determination of a threshold efficiency. J. Dairy Sci. 104:1759–1776. https://doi.org/10.3168/jds.2020-19205.

Lee, C., A. N. Hristov, T. W. Cassidy, K. S. Heyler, H. Lapierre, G. A. Varga, M. J. de Veth, R. A. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. J. Dairy Sci. 95:6042–6056. https://doi.org/10.3168/jds.2012-5581.

Liu, C., D. J. Schingoethe, and G. A. Stegeman. 2000. Corn distillers grains versus a blend of protein supplements with or without ruminally protected amino acids for lactating dairy cows. J. Dairy Sci. 83:2075–2084. https://doi.org/10.3168/jds.S0022-0302(00)75089-2.

Ludden, P. A., T. L. Wechter, and B. W. Hess. 2002. Effects of oscillating dietary protein on nutrient digestibility, nitrogen metabolism, and gastrointestinal organ mass in sheep. J. Anim. Sci. 80:3021–3026. https://doi.org/10.2527/2002.80113021x.

Mepham, T. B. 1987. The composition of milks. Pages 30–49 in Physiology of Lactation. Open University Press.

Morris, D. L., and P. J. Kononoff. 2020. Effects of rumen-protected lysine and histidine on milk production and energy and nitrogen utilization in diets containing hydrolyzed feather meal fed to lactating Jersey cows. J. Dairy Sci. 103:7110–7123. https://doi.org/10.3168/jds.2020-18368.

Nichols, J. R., D. J. Schingoethe, H. A. Maiga, M. J. Brouk, and M. S. Piepenbrink. 1998. Evaluation of corn distillers grains and ruminally protected lysine and methionine for lactating dairy cows. J. Dairy Sci. 81:482–491. https://doi.org/10.3168/jds.S0022-0302(98)75600-0.

Nichols, K., A. Bannink, and J. Dijkstra. 2019a. Energy and nitrogen balance of dairy cattle as affected by provision of different essential amino acid profiles at the same metabolizable protein supply. J. Dairy Sci. 102:8963–8976. https://doi.org/10.3168/jds.2019-16400.

Nichols, K., A. Bannink, J. Doelman, and J. Dijkstra. 2019b. Mammary gland metabolite utilization in response to exogenous glucose or long-chain fatty acids at low and high metabolizable protein levels. J. Dairy Sci. 102:7150–7167. https://doi.org/10.3168/jds.2019-16285.

Nichols, K., A. Bannink, S. Pacheco, H. J. van Valenberg, J. Dijkstra, and H. van Laar. 2018. Feed and nitrogen efficiency are affected differently but milk lactose production is stimulated equally when isoenergetic protein and fat is supplemented in lactating dairy cow diets. J. Dairy Sci. 101:7857–7870. https://doi.org/10.3168/jds.2017-14276.

Nichols, K., J. Dijkstra, M. J. H. Breuer, S. Lemosquet, W. J. J. Gerrits, and A. Bannink. 2022. Essential amino acid profile of supplemental metabolizable protein affects mammary gland metabolism and whole-body glucose kinetics in dairy cattle. J. Dairy Sci. 105:7354–7372. https://doi.org/10.3168/jds.2021-21576.

Nichols, K., J. Dijkstra, H. van Laar, S. Pacheco, H. J. van Valenberg, and A. Bannink. 2019c. Energy and nitrogen partitioning in dairy cows at low or high metabolizable protein levels is affected differently by postrumen glucogenic and lipogenic substrates. J. Dairy Sci. 102:395–412. https://doi.org/10.3168/jds.2018-15249.

Nichols, K., J. J. M. Kim, M. Carson, J. A. Metcalf, J. P. Cant, and J. Doelman. 2016. Glucose supplementation stimulates peripheral branched-chain amino acid catabolism in lactating dairy cows during essential amino acid infusions. J. Dairy Sci. 99:1145–1160. https://doi.org/10.3168/jds.2015-9912.

Nichols, K., R. Rauch, L. Lippens, D. J. Seymour, and J. Martín-Tereso. 2023. Dose response to postruminal urea in lactating dairy cattle. J. Dairy Sci. 106:8694–8709. https://doi.org/10.3168/jds.2023-23402.

Niu, M., Y. Ying, P. A. Bartell, and K. J. Harvatine. 2014. The effects of feeding time on milk production, total-tract digestibility, and daily rhythms of feeding behavior and plasma metabolites and hormones in dairy cows. J. Dairy Sci. 97:7764–7776. https://doi.org/10.3168/jds.2014-8261.

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

Owen, O. E., S. C. Kalhan, and R. W. Hanson. 2002. The key role of anaplerosis and cataplerosis for citric acid cycle function. J. Biol. Chem. 277:30409–30412. https://doi.org/10.1074/jbc.R200006200.

Pereira, A. B. D., D. C. Moura, N. L. Whitehouse, and A. F. Brito. 2020. Production and nitrogen metabolism in lactating dairy cows fed finely ground field pea plus soybean meal or canola meal with or without rumen-protected methionine supplementation. J. Dairy Sci. 103:3161–3176. https://doi.org/10.3168/jds.2019-17186.

Räisänen, S. E., C. F. A. Lage, C. Zhou, A. Melgar, T. Silvestre, D. E. Wasson, S. F. Cueva, J. Werner, T. Takagi, M. Miura, and A. N. Hristov. 2022. Lactational performance and plasma and muscle amino acid concentrations in dairy cows fed diets supplying 2 levels of digestible histidine and metabolizable protein. J. Dairy Sci. 105:170–187. https://doi.org/10.3168/jds.2021-20800.

Rauch, R., J. Martín-Tereso, J. B. Daniel, and J. Dijkstra. 2021. Dietary protein oscillation: Effects on feed intake, lactation performance, and milk nitrogen efficiency in lactating dairy cows. J. Dairy Sci. 104:10714–10726. https://doi.org/10.3168/jds.2021-20219.

Rauch, R., K. Nichols, J. B. Daniel, J. Martín-Tereso, and J. Dijkstra. 2023. Dietary protein oscillation: effects on digestibility, nutrient balance and estimated microbial protein synthesis in lactating dairy cows. Animal 17:100695. https://doi.org/10.1016/j.animal.2022.100695.

Rottman, L. W., Y. Ying, K. Zhou, P. A. Bartell, and K. Harvatine. 2014. The daily rhythm of milk synthesis is dependent on the timing of feed intake in dairy cows. Physiol. Rep. 2:e12049. https://doi.org/10.14814/phy2.12049.

Salfer, I. J., and K. J. Harvatine. 2020. Night-restricted feeding of dairy cows modifies daily rhythms of feed intake, milk synthesis and plasma metabolites compared with day-restricted feeding. Br. J. Nutr. 123:849–858. https://doi.org/10.1017/S0007114520000057.

Santos, F. A. P., J. E. P. Santos, C. B. Theurer, and J. T. Huber. 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci. 81:3182–3213. https://doi.org/10.3168/jds.S0022-0302(98)75884-9.

Sarwar, G., and H. G. Botting. 1990. Rapid analysis of nutritionally important free amino acids in serum and organs (liver, brain, and heart) by liquid chromatography of precolumn phenylisothiocyanate derivatives. J. Assoc. Off. Anal. Chem. 73:470–475. https://doi.org/10.1093/jaoac/73.3.470.

Satter, L. D. 1986. Protein supply from undegraded protein. J. Dairy Sci. 69:2734–2749. https://doi.org/10.3168/jds.S0022-0302(86)80722-6.

Schwab, C. G., and G. A. Broderick. 2017. A 100-year review: Protein and amino acid nutrition in dairy cows. J. Dairy Sci. 100:10094–10112. https://doi.org/10.3168/jds.2017-13320.

Seymour, W. M., C. E. Polan, and J. H. Herbein. 1990. Effects of dietary protein degradability and casein or amino acid infusions on production and plasma amino acids in dairy cows. J. Dairy Sci. 73:735–748. https://doi.org/10.3168/jds.S0022-0302(90)78727-9.

Sherwood, A. 2000. Amino acid measurement in body fluids using PITC derivatives. Pages 169–175 in Amino Acid Analysis Protocols. C. Cooper, N. Packer, and K. Williams, ed. Humana Press Inc., Totowa, NJ. https://doi.org/10.1385/1-59259-047-0:169.

Spackman, D. H., E. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30:1190–1206. https://doi.org/10.1021/ac60139a006.

Spanghero, M., and Z. M. Kowalski. 2021. Updating analysis of nitrogen balance experiments in dairy cows. J. Dairy Sci. 104:7725–7737. https://doi.org/10.3168/jds.2020-19656.

Stevens, A. V., K. Karges, P. Rezamand, A. H. Laarman, and G. E. Chibisa. 2021. Production performance and nitrogen metabolism in dairy cows fed supplemental blends of rumen undegradable protein and rumen-protected amino acids in low- compared with high-protein diets containing corn distillers grains. J. Dairy Sci. 104:4134–4145. https://doi.org/10.3168/jds.2020-19697.

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.

Van den Bossche, T., K. Goossens, B. Ampe, G. Haesaert, J. De Sutter, J. L. De Boever, and L. Vandaele. 2023. Effect of supplementing rumen-protected methionine, lysine, and histidine to low-protein diets on the performance and nitrogen balance of dairy cows. J. Dairy Sci. 106:1790–1802. https://doi.org/10.3168/jds.2022-22041.

Van Es, A. J. H. 1978. Feed evaluation for ruminants. I. The systems in use from May 1977-onwards in the Netherlands. Livest. Prod. Sci. 5:331–345. https://doi.org/10.1016/0301-6226(78)90029-5.

van Gastelen, S., E. C. Antunes-Fernandes, K. A. Hettinga, G. Klop, S. J. J. Alferink, W. H. Hendriks, and J. Dijkstra. 2015. Enteric methane production, rumen volatile fatty acid concentrations, and milk fatty acid composition in lactating Holstein-Friesian cows fed grass silage- or corn silage-based diets. J. Dairy Sci. 98:1915–1927. https://doi.org/10.3168/jds.2014-8552.

Varvikko, T., A. Vanhatalo, T. Jalava, and P. Huhtanen. 1999. Lactation and metabolic responses to graded abomasal doses of methionine and lysine in cows fed grass silage diets. J. Dairy Sci. 82:2659–2673. https://doi.org/10.3168/jds.S0022-0302(99)75523-2.

Warner, D., J. Dijkstra, S. Tamminga, and W. F. Pellikaan. 2013. Passage kinetics of concentrates in dairy cows measured with carbon stable isotopes. Animal 7:1935–1943. https://doi.org/10.1017/S1751731113001584.

Warner, D., S. C. Podesta, B. Hatew, G. Klop, H. van Laar, A. Bannink, and J. Dijkstra. 2015. Effect of nitrogen fertilization rate and regrowth interval of grass herbage on methane emission of zero-grazing lactating dairy cows. J. Dairy Sci. 98:3383–3393. https://doi.org/10.3168/jds.2014-9068.

Wu, S. H. W., and A. Papas. 1997. Rumen-stable delivery systems. Adv. Drug Deliv. Rev. 28:323–334. https://doi.org/10.1016/S0169-409X(97)00087-2.

Yoder, P. S., X. Huang, I. A. Teixeira, J. P. Cant, and M. D. Hanigan. 2020. Effects of jugular infused methionine, lysine, and histidine as a group or leucine and isoleucine as a group on production and metabolism in lactating dairy cows. J. Dairy Sci. 103:2387–2404. https://doi.org/10.3168/jds.2019-17082.

Zang, Y., L. H. P. Silva, Y. C. Geng, M. Ghelichkhan, N. L. Whitehouse, M. Miura, and A. F. Brito. 2021. Dietary starch level and rumen-protected methionine, lysine, and histidine: Effects on milk yield, nitrogen, and energy utilization in dairy cows fed diets low in metabolizable protein. J. Dairy Sci. 104:9784–9800. https://doi.org/10.3168/jds.2020-20094.

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