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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

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ABSTRACT

Our objective was to investigate the interactions between starch level and rumen-protected Met, Lys, His (RP-MLH) on milk yield, plasma AA concentration, and nutrient utilization in dairy cows fed low metabolizable protein diets (mean = −119 g/d of metabolizable protein balance). Sixteen multiparous Holstein cows (138 ± 46 d in milk, 46 ± 6 kg/d in milk) were used in a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments. Each period lasted 21 d with 14 d for diet adaptation and 7 d for data and sample collection. Dietary starch level varied by replacing (dry matter basis) pelleted beet pulp and soyhulls with ground corn resulting in the following treatments: (1) 20% pelleted beet pulp and 10% soyhulls (reduced starch = RS), (2) RS plus RP-MLH (RS+AA), (3) 30% ground corn (high starch = HS), and (4) HS plus RP-MLH (HS+AA). Dietary starch concentrations averaged 12.3 and 34.4% for RS and HS basal diets, respectively. Diets were supplemented with RP-MLH products to supply digestible Met, Lys, and His. Compared with RS and RS+AA diets, HS and HS+AA diets increased yields of milk (37.9 vs. 40.1 kg/d) and milk protein (1.07 vs. 1.16 kg/d) and decreased dry matter intake (25.9 vs. 25.2 kg/d), milk urea N (12.6 vs. 11.0 mg/dL), and plasma urea N (13.3 vs. 11.6 mg/dL). Milk N efficiency was greater in cows fed the HS and HS+AA than RS and RS+AA diets (28.9 vs. 25%), and RP-MLH supplementation improved milk true protein concentration. Starch level × RP-MLH interactions were observed for plasma concentrations of Arg and Lys, with RP-MLH being more effective to increase plasma Arg (+16%) and Lys (+23%) when supplemented to the RS than the HS basal diet. Replacing pelleted beet pulp and soyhulls with ground corn lowered the plasma concentrations of all essential AA except Met and Thr. In addition, the plasma concentrations of His and Met increased with RP-MLH. The apparent total-tract digestibilities of neutral and acid detergent fiber were lower, and those of starch and ether extract greater in cows offered the HS and HS+AA diets than RS and RS+AA diets. Urinary excretion of urea N decreased by replacing pelleted beet pulp and soyhulls with ground corn. Enteric CH_4 production, CH_4 yield, and CH_4 intensity all decreased in the HS and HS+AA versus RS and RS+AA diets. Diets did not affect the intakes of gross energy, metabolizable energy, and net energy of lactation. In contrast, digestible energy intake increased with feeding the RS and RS+AA diets, whereas CH_4 energy decreased in cows fed the HS and HS+AA diets. Supplementation with RP-MLH had no effect on energy utilization variables. Overall, the lack of interactions between dietary starch level and RP-MLH supplementation on most variables measured herein showed that the effects of starch intake and RP-MLH were independent or additive.

Key words: essential amino acid, fibrous byproduct, ground corn, methane

INTRODUCTION

Previous research revealed that yields of milk and milk protein decreased in dairy cows fed MP-deficient versus MP-adequate diets (Lee et al., 2012; Giallongo et al., 2016). It was also shown that supplementation of MP-deficient diets with rumen-protected Met, Lys, His (**RP-MLH**) restored milk and milk protein yields to the same levels observed with MP-adequate diets (Lee et al., 2012; Giallongo et al., 2016), thus confirming

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that Met, Lys, and His limited production responses. However, these previous studies (Lee et al., 2012; Giallongo et al., 2016) did not explore whether MP interacts with energy to modulate the synthesis of milk and milk protein. Rius et al. (2010b) reported a 21% increase in milk protein yield in dairy cows fed high (mean = 1.54 Mcal/kg of DM) versus low (mean = 1.45 Mcal/kg of DM) energy diets independent of MP supply, which agree with results obtained by Omphalius et al. (2019). In contrast, Rius et al. (2010a) observed that milk protein yield increased in response to abomasal infusion of casein in the presence of starch, characterizing an energy by protein interaction. Despite key information provided by Rius et al. (2010a,b) and Omphalius et al. (2019), we are not aware of any investigation that has focused on the potential interactions between starch intake and RP-MLH supplementation on milk protein yield and nutrient utilization in dairy cows fed low MP diets.

Rulquin and Delaby (1997) investigated whether dietary levels of energy (87 or 100% of requirements) and RP-Met supplementation would interact to modulate treatment effects on milk yield, milk composition, and plasma concentrations of several metabolites including AA. No significant interactions were observed for most variables analyzed, indicating that, overall, responses to treatments were independent and additive (Rulquin and Delaby, 1997). Nevertheless, further research is needed to better understand potential energy by RP-AA interactions, which underpin processes that affect N and energy utilization in lactating dairy cows. Specifically, we are not aware of any published study evaluating the effects of different dietary starch levels obtained with replacing ground corn for nonforage fiber sources (**NFFS**) supplemented or not with RP-MLH on milk yield and nutrient utilization in dairy cows fed low MP diets. Boerman et al. (2015) reported that yields of milk and milk protein decreased when ground corn substituted soyhulls at 30% of the diet DM, thus showing that reduced energy intake limited production responses.

We hypothesized that compared with either increased dietary starch level or RP-MLH supplementation, these feeding strategies together could interact to improve yields of milk and milk components and N and energy utilization in dairy cows fed low MP diets. Our objective was to investigate the interrelationships between dietary starch level, varied by replacing pelleted beet pulp and soyhulls with ground corn, and RP-MLH supplementation on yields of milk and milk components, plasma AA concentration, apparent total-tract digestibility of nutrients, enteric CH_4 emission, and N and energy utilization in dairy cows offered low MP diets.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee (protocol no. 180305) of the University of New Hampshire (Durham). The experiment was conducted at the University of New Hampshire Fairchild Dairy Teaching and Research Center (Durham) from June 11 to August 26, 2018.

Cows, Experimental Design, and Treatments

Sixteen multiparous Holstein cows averaging (mean ± SD) 138 ± 46 DIM, 46 ± 6 kg/d of milk, and 700 ± 55 kg of BW at the beginning of the study were selected. Animals were housed in a tiestall barn equipped with water bowls for free access to water and feed tubs for individual feeding. Cows were milked twice per day at 0530 and 1630 h, with milk yield recorded at each milking throughout the experiment. Animals were weighed (Northeast Scale Co.) immediately after the afternoon milking during 3 consecutive days before the beginning of the study and at the end of each period to compute BW change. Body condition score was assigned by 3 trained individuals before the start of the experiment and on the last day of each period following the procedures outlined by Wildman et al. (1982). Dietary ingredients were mixed and offered as TMR twice daily at 0600 and 1700 h using a Super Data Ranger mixer (American Calan Inc.). Orts were collected and weighed daily before the afternoon feeding. Feed offered was adjusted daily to achieve 5 to 10% orts, with individual intake recorded for the duration of the experiment.

Cows were blocked by milk yield and randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. Squares were balanced for potential first-order carryover effects in subsequent periods as each treatment immediately preceded and followed each other exactly once in every square (Williams, 1949). Each experimental period lasted 21 d, including 14 d for diet adaptation and 7 d for data and sample collection. Dietary starch level varied by replacing (DM basis) pelleted beet pulp and soyhulls with ground corn, resulting in the following treatments: (1) 20% pelleted beet pulp and 10% soyhulls (reduced starch = **RS**), (2) RS plus RP-MLH (**RS+AA**), (3) 30% ground corn (high starch = **HS**), and (4) HS plus RP-MLH (**HS+AA**). The basal diets were formulated (NRC, 2001) to be isonitrogenous and meet nutritional requirements, except MP, of a lactating dairy cow averaging 700 kg of BW, 138 DIM, 42 kg/d of milk, 3.5% milk fat, 3% milk true

protein, 4.8% milk lactose, and 25 kg/d of DMI and contained (DM basis) 35.7% corn silage, 14.7% mixed (mostly grass) haylage, and 49.6% concentrate. The RP-MLH supplements were top-dressed to the TMR to meet the requirements of digestible MLH in grams per day (Schwab et al., 2005). The amounts of RP-Met (Smartamine M; Adisseo USA Inc.), RP-Lys (AjiPro-L; Ajinomoto Health & Nutrition North America Inc.), and RP-His (Ajinomoto prototype supplement; Ajinomoto Co. Inc.) supplemented averaged 25, 76, and 110 g/d, respectively. The RP-MLH supplements contained 75% DL-Met (80% bioavailability; Chirgwin et al., 2015), 40% Lys (54% bioavailability; Giallongo et al., 2016), and 44% His (14% bioavailability, according to the manufacturer), and were expected to provide 15, 16, and 6.78 g/d of digestible Met, Lys, and His, respectively. The nutritional and AA composition of individual dietary ingredients are shown in Tables 1 and 2, respectively. The ingredient and nutritional composition of the basal diets are presented in Table 3, and the NRC (2001) evaluation of the 4 treatments is shown in Table 4.

Feed Sampling and Analyses

Corn silage, mixed (mostly grass) haylage, TMR, and orts were collected thrice weekly and composited by week. Composite samples were dried (55°C, 48 h) in a forced-air oven (VWR Scientific) for determination of DM to adjust the TMR, on an as fed basis, and to calculate DMI throughout the experiment. Samples of forages, concentrates, TMR, and orts were collected thrice during the sampling phase of each period and pooled by week. Weekly ingredients, TMR, and orts were lyophilized for 48 h (Labconco Inc.), ground with a Wiley mill (A. H. Thomas Co.) to pass through a 1-mm screen, and stored in air-tight glass jars until nutritional analysis.

Lyophilized ground samples of dietary ingredients were shipped to Dairy One Cooperative Inc. (Ithaca, NY) and analyzed for DM, CP, soluble CP, NDF, ADF, ADL, starch, ether extract, ash, and individual minerals by wet chemistry and chromatographic procedures (https://dairyone.com/download/forage-forage-lab-analytical-procedures). In addition, TMR and orts were analyzed for CP, NDF, ADF, gross energy (**GE**; IKA C2000 basic calorimeter system; KA Works Inc.), and ash at Dairy One Cooperative Inc. Samples of dietary ingredients were ground (0.5-mm screen) and analyzed for AA by cation exchange chromatography coupled with postcolumn ninhydrin derivatization using norleucine as the internal standard (method 982.30; AOAC International, 2016) at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO). Tryptophan was determined after alkaline hydrolysis, and sulfur AA were analyzed

Table 1. Nutrient composition of ingredients used in the experimental diets (% of DM, unless otherwise noted)

Item	Corn silage	Haylage[1]	Ground corn	Pelleted beet pulp	Soyhulls	Soybean meal	Canola meal	DDGS[2]	Urea
No. of samples	4	2	2	2	2	3	3	3	3
DM, % of fresh matter	29.4	28.5	85.6	86.7	89.4	85.9	85.5	83.5	98.9
CP	8.18	16.9	8.20	8.55	11.8	53.8	41.9	31.7	283
Soluble CP, % of CP	66.5	47.5	26.0	14.5	29.5	21.7	20.0	17.7	NA[3]
NDF	43.5	53.8	8.25	35.5	61.0	9.40	29.0	34.9	NA
ADF	24.9	36.3	2.80	22.6	44.8	7.77	20.1	15.7	NA
ADL	2.85	6.05	0.95	5.30	2.15	0.97	8.07	4.40	NA
Starch	33.3	0.75	74.5	0.20	1.85	0.30	0.57	1.40	NA
Ether extract	3.33	4.45	3.75	1.20	2.45	1.57	5.00	14.1	NA
NE_L, Mcal/kg of DM	1.62	1.30	2.06	1.31	1.52	1.83	1.63	2.12	NA
Ash	3.81	9.20	2.18	13.6	5.68	7.99	7.01	7.45	NA
Ca	0.15	0.66	0.01	1.43	0.52	0.28	0.61	0.03	NA
P	0.30	0.40	0.33	0.11	0.15	0.82	1.10	1.11	NA
Mg	0.15	0.26	0.10	0.26	0.23	0.25	0.52	0.31	NA
K	0.95	2.72	0.39	0.36	1.48	2.11	1.08	1.17	NA
Na	0.01	0.06	0.01	0.03	0.01	0.00	0.06	0.09	NA
S	0.10	0.27	0.10	0.25	0.14	0.44	0.80	0.44	NA
Fe, mg/kg of DM	335	315	35.0	2,210	491	85.0	128	120	NA
Zn, mg/kg of DM	24.8	31.5	18.0	32.0	56.0	41.7	54.0	63.7	NA
Cu, mg/kg of DM	6.00	10.5	2.00	9.50	8.00	12.7	5.33	8.00	NA
Mn, mg/kg of DM	14.3	47.5	4.00	102	18.0	30.3	58.3	19.3	NA
Mo, mg/kg of DM	1.73	3.85	0.75	1.20	0.95	4.60	1.37	1.53	NA

[1]Mixed (mostly grass) haylage.

[2]DDGS = corn dried distillers grains with solubles.

[3]NA = not analyzed.

after performic acid oxidation (method 988.15; AOAC International, 2016).

Milk and Blood Sampling and Analyses

Milk samples were collected using automatic samplers during 4 consecutive milkings starting in the afternoon milking of d 15 of each period. Milk samples were transferred into tubes preserved with 2-bromo-2-nitropropane-1,3 diol (Broad Spectrum Microtabs II; Advanced Instruments Inc.) and stored at 4°C until shipped overnight to Dairy One Cooperative Inc. laboratory for determination of fat, true protein, lactose, and MUN by Fourier transform infrared spectroscopy using a MilkoScan FT+ (Foss Inc.).

Blood samples were collected into vacutainer 15% EDTA tubes (Monoject) from the coccygeal vessels approximately 4 h after the morning feeding on d 16 and 17 of each period. Tubes were immediately placed in a chill bucket with beads (Chemglass Life Sciences) and transported to the laboratory for centrifugation (2,155 × *g*, 20 min, 4°C) using an Eppendorf centrifuge (model 5810; Eppendorf). Plasma samples were composited by cow and period, and the composite samples were used to analyze the concentrations of AA, His-containing metabolites, and urea N (**PUN**) at Ajinomoto Co. Inc. using a High-Speed AA analyzer L-8900 (Hitachi High-Technologies Co.) following the procedures reported by the manufacturer (https://www.hitachi-hightech.com/us/library/literature/brochure-l-8900-amino-acid-analyzer.html). Codified plasma samples were shipped to Ajinomoto Co. Inc. to blind treatments identity. Whole blood samples for hemoglobin analysis were taken on d 16 of each period as reported above and shipped overnight to the Cornell University Animal Health Diagnostic Center (Ithaca, NY) on the same day of collection. Hemoglobin was analyzed via spectrophotometry using lysed red blood cells, with free hemoglobin converted to cyanmethemoglobin after cyanide addition and detection set at 540 nm wavelength.

Fecal and Urinary Sampling and Analyses

Fecal grab samples were taken directly from the rectum or during voluntary defecation at 0600 and 1500 h (d 18); 0900, 1200, and 1800 h (d 19); and 0000, 0300, and 2100 h (d 20) of the sampling phase in each period. Fecal samples (~200 g/sampling) were collected into 100-mL specimen containers and transferred into 4-L plastic bags to generate composited samples (wet weight) by cow per period. Next, samples were dried in a forced-air oven (VWR Scientific) at 55°C for ap-

Table 2. Amino acid composition of ingredients used in the experimental diets (n = 1 composited sample per feedstuff)

Item	Corn silage	Haylage[1]	Ground corn	Pelleted beet pulp	Soyhulls	Soybean meal	Canola meal	DDGS[2]
Total AA, g/100 g	5.71	10.2	7.31	6.98	10.7	50.1	35.9	27.3
EAA, % of total AA								
Arg	1.93	3.25	4.79	2.58	5.03	7.40	6.25	4.69
His	1.40	1.67	2.87	3.44	2.80	2.63	2.87	2.78
Ile	4.55	5.31	3.56	4.87	4.29	4.87	4.49	4.25
Leu	11.6	9.15	11.8	7.59	7.08	7.82	7.50	11.5
Lys	2.63	4.82	3.28	4.01	7.08	6.52	6.22	3.44
Met	1.75	1.77	1.78	1.86	1.12	1.32	2.20	1.90
Phe	4.55	5.91	4.92	4.87	4.29	5.29	4.44	5.34
Thr	3.50	4.63	3.69	5.30	3.82	3.91	4.63	4.03
Trp	0.53	1.08	0.82	0.72	0.75	1.38	1.31	0.81
Val	5.95	6.79	4.65	7.31	4.85	4.87	5.47	5.16
NEAA, % of total AA								
Ala	12.4	9.45	7.39	5.59	4.47	4.35	4.66	6.99
Asp	5.60	9.06	6.70	8.74	9.69	11.3	7.34	6.63
Cys	1.58	1.08	2.33	1.43	1.96	1.42	2.79	2.23
Gly	5.08	5.91	3.97	5.16	7.83	4.27	5.44	4.32
Glu	12.3	9.15	17.8	10.5	12.0	18.4	18.4	16.6
Hydroxylysine	6.30	5.02	0.41	0.43	0.75	0.16	0.53	0.48
Hydroxyproline	0.35	0.69	0.27	6.30	4.38	0.12	1.03	1.10
Orn	0.53	0.89	0.00	0.14	0.09	0.06	0.03	0.11
Pro	8.23	6.30	8.62	5.73	5.87	5.37	6.92	8.75
Ser	3.50	3.84	4.65	4.73	5.13	4.55	4.13	4.72
Try	2.28	2.95	2.74	4.30	4.10	3.79	3.04	3.84
Taurine	3.50	1.28	3.01	4.44	2.61	0.26	0.36	0.33

[1]Mixed (mostly grass) haylage.

[2]DDGS = corn dried distillers grains with solubles.

proximately 72 h and ground to pass through a 1-mm screen (Wiley mill; A. H. Thomas Co.). Fecal samples were analyzed for DM, CP, NDF, ADF, GE, and ash at Dairy One Cooperative Inc. Triplicate samples (~0.5 g) of feces, TMR, concentrates, and orts were weighed into Ankom F57 bags (25 μm pore size; Ankom Technology), placed in a larger laundry nylon bag, and inserted in the rumen of 1 ruminally cannulated, late-lactation Holstein cow fed a 50:50 forage:concentrate ratio TMR containing corn silage and mixed (mostly grass) haylage (12-d incubation). After removal from the rumen, bags were rinsed with tap water and analyzed in-house for NDF using an Ankom^{2000} fiber analyzer [Ankom Technology method 6; solutions as in (Van Soest et al., 1991)]. Indigestible NDF was used as the internal marker to estimate fecal output of DM and apparent total-tract digestibility of nutrients (Cochran et al., 1986; Huhtanen et al., 1994).

Table 3. Ingredient and nutritional composition (% of DM, unless otherwise noted) of the experimental diets

Item	Basal diets	
	Reduced starch	High starch
Ingredient		
Corn silage	35.7	35.7
Mixed (mostly grass) haylage	14.7	14.7
Ground corn	—	30.0
Pelleted beet pulp	20.0	—
Soyhulls	10.0	—
Soybean meal	8.71	8.71
BergaFat F100[1]	3.00	3.00
Canola meal	2.76	2.76
Mineral and vitamins premix[2]	2.50	2.50
Sodium bicarbonate	1.00	1.00
DDGS[3]	0.92	0.92
Urea	0.70	0.70
Nutrient composition		
DM, % of fresh matter	46.8	46.8
CP	16.4	16.0
NDF	38.6	27.9
Forage NDF	23.4	23.4
ADF	24.6	16.4
ADL	3.53	2.54
Starch	12.3	34.4
Ether extract	5.70	6.40
NE_L,[4] Mcal/kg of DM	1.59	1.68
Ca	1.00	0.60
P	0.40	0.40
Digestible His,[4] % of MP	2.19	2.13
Digestible Met,[4] % of MP	1.83	1.87
Digestible Lys,[4] % of MP	6.63	6.71
Gross energy, Mcal/kg of DM	4.16	4.25

[1]BergaFat F100 is a rumen-stable fat containing 80% palmitic acid (Berg+Schmidt America LLC).

[2]Mineral and vitamin premix contained (DM basis): 17.2% Ca, 1.46% P, 5.71% Mg, 8.53% Cl, 0.12% K, 14.7% Na, 0.44% S, 28 mg/kg of Co, 326 mg/kg of Cu, 2,380 mg/kg of Fe, 973 mg/kg of Mn, 1,480 mg/kg of Zn, 2,500 kIU/kg of vitamin A, 400 kIU/kg of vitamin D, and 1 kIU/kg of vitamin E.

[3]DDGS = corn dried distillers grains with solubles.

[4]Estimated using the NRC (2001) model with actual DMI, animal variables (DIM, lactation number, and BW), nutrient composition of dietary ingredients, and milk yield and milk composition before the experiment began.

Spot urine samples were collected concurrently with fecal samples into 100-mL specimen containers through stimulation of the pudendal nerve by massaging the area below the vulva or during voluntary urination. After each sampling, 1 mL of urine was pipetted into 50-mL centrifuge tubes containing 32 mL of 0.072 *N* H_2SO_4 to obtain composited urine samples by cow per period and stored at −20°C until analyses. After thawing at room temperature, samples were analyzed for concentrations of creatinine (assay kit no. 500701, Cayman Chemical Co.) using a chromate microplate reader set at a wavelength of 492 nm (Awareness Technology Inc.), allantoin (Chen et al., 1992), uric acid (assay kit no. 1045–225; Stanbio Laboratory), urea N (Stanbio Urea Nitrogen Kit 580; Stanbio Laboratory Inc.), and total N (micro-Kjeldahl analysis, AOAC, 1990; Dairy One Cooperative Inc.). Allantoin, uric acid, and urea N were determined at wavelengths of 522, 520, and 520 nm, respectively, with a UV-visible spectrophotometer (Beckman Coulter Inc.). Daily urine volume was estimated from urinary creatinine concentration assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999), with the average BW measured in the last 3 d of each period used in the calculations. Urinary excretion of urea N, total N, allantoin, uric acid, and total purine derivatives (allantoin plus uric acid) were calculated by multiplying the concentration of each of these metabolites by the urinary volume.

Measurements of Gaseous Fluxes

Emissions of CO_2 and enteric CH_4 were measured at 0200 and 1400 h (d 15), 0500 and 1700 h (d 16), 0800 and 2000 h (d 17), and 1100 and 2300 h (d 18) of the sampling phase in each period using the GreenFeed system (C-Lock Inc.) following a sampling schedule similar to that reported by Harper et al. (2017). The GreenFeed unit was placed in front of each cow for approximately 5 min to sample breath and eructated gases, and then moved to the barn alley for about 2 min to sample background gases. The actual gaseous sampling time recorded by the GreenFeed unit averaged 5 min and 14 s, with the unit moved from a cow to the next cow sequentially, which took about 2 h to complete the gaseous sampling. Cows were trained to access to the GreenFeed unit for 2 wk before the beginning of the study. A soybean meal-NFFS-based bait pellet (Hi-Line 16% Dairy/Beef Pellet; Poulin Grain Inc.) with (DM basis) 19.6% CP, 35% NDF, 15.2% ADF, 16.7% starch, 6.2% ether extract, and 4.56 Mcal/kg of GE was used

during training and samplings. Approximately 25 g of bait pellets (as fed) were dropped every 15 s during each gaseous sampling, resulting in 0.44 kg of bait DM consumed per cow per time point. One cow did not consistently access the GreenFeed unit, and her data were excluded from the statistical analyses. A complete description of the gaseous sampling protocol and emission calculations was reported by Dorich et al. (2015).

Calculations

Yields of milk components were calculated using milk yield and concentrations of milk components obtained from samples collected during milkings from d 15 to 17, summed for daily total, and averaged by period. Energy loss as CH_4 was calculated by multiplying enteric CH_4 production (L/d) by its enthalpy (9.45 kcal/L). Both digestible energy (**DE**) and ME intakes were calculated as follows:

$$\text{DE intake (Mcal/d)} = \text{GE intake (Mcal/d)} - \text{fecal energy (Mcal/d)};$$

$$\text{ME intake (Mcal/d)} = \text{DE intake (Mcal/d)} - \text{urinary energy (Mcal/d)} - CH_4 \text{ energy (Mcal/d)},$$

where urinary energy was calculated using the following equation: urinary energy (Mcal/d) = [14.6 × urinary N output (g/d)]/1,000 (Morris et al., 2021).

Table 4. NRC (2001) evaluation of experimental diets with different starch levels supplemented or not with rumen-protected Met, Lys, and His (RP-MLH[1])

	Treatment[3]			
Item[2]	RS	RS+AA	HS	HS+AA
NE_L, Mcal/d				
Requirement	40.0	40.0	40.0	40.0
Supply	40.1	40.4	41.4	41.5
Balance	0.10	0.40	1.30	1.40
MP, g/d				
Requirement	2,773	2,780	2,743	2,747
Supply	2,666	2,727	2,564	2,612
Balance	−107	−53	−180	−135
RDP, g/d				
Requirement	2,479	2,497	2,539	2,548
Supply	2,615	2,634	2,623	2,633
Balance	136	137	84	85
RUP, g/d				
Requirement	1,675	1,669	1,544	1,541
Supply	1,537	1,590	1,321	1,366
Balance	−137	−79	−223	−175
Digestible His, g/d				
Requirement[4]	61	61	60	60
Supply from diet	58	59	55	55
Supply from RP-His	0	7	0	7
Balance	−3	5	−5	2
Digestible Met, g/d				
Requirement[4]	61	61	60	60
Supply from diet	49	49	48	48
Supply from RP-Met	0	15	0	15
Balance	−12	3	−12	3
Digestible Lys, g/d				
Requirement[4]	183	181	181	181
Supply	177	178	172	173
Supply from RP-Lys	0	16	0	16
Balance	−7	13	−9	8

[1]RP-MLH = 25 g/d of RP-Met (Smartamine M; Adisseo USA Inc.), 76 g/d of RP-Lys (AjiPro-L; Ajinomoto Health & Nutrition North America Inc.), and 110 g/d of RP-His (prototype supplement; Ajinomoto Co. Inc.).

[2]All values were estimated using the NRC (2001) model with actual DMI, animal variables (DIM, lactation number, and BW), nutrient composition of dietary ingredients, and milk yield and milk composition before the experiment began.

[3]RS (reduced starch, 12.3% starch) = diet containing 20% pelleted beet pulp and 10% soyhulls, RS+AA = RS diet supplemented with RP-MLH, HS (high starch, 34.4% starch) = diet containing 30% ground corn, and HS+AA = HS diet supplemented with RP-MLH.

[4]Requiements of digestible His, Met, and Lys were calculated as 2.2, 2.2, and 6.6% of MP requirements, respectively (Schwab et al., 2005).

Tissue energy was calculated as reported by Morris and Kononoff (2020) as follows:

$$\text{Tissue energy (Mcal/d)} = \text{ME intake (Mcal/d)} - \text{heat production (Mcal/d)} - \text{milk energy (Mcal/d)},$$

where heat production (Mcal/d) = [0.0185 × CO_2 (L/d) + 6.8]/4.184 (Bayat et al., 2019), and milk energy (Mcal/d) = [(0.0929 × milk fat%) + (0.0563 × milk true protein %) + (0.0395 × milk lactose %)] × milk yield (kg/d) (NRC, 2001). Tissue energy was corrected to an NE_L basis for calculation of NE_L as follows:

$$\text{Tissue energy (Mcal of NE}_L\text{/d)} = \text{positive tissue energy} \times k_G/k_T \text{ or negative tissue energy} \times k_T,$$

where k_G = efficiency of utilizing ME intake for tissue gain, and k_T = efficiency of utilizing body reserve energy for milk yield. Efficiency coefficient values of 0.70 and 0.89 were used for k_G and k_T, respectively (Moraes et al., 2015). Calculation of NE_L was done according to the NRC (2001) using the following equation:

$$NE_L \text{ (Mcal/d)} = 0.080 \times BW^{0.75} + \text{milk energy (Mcal/d)} + \text{tissue energy (Mcal/d)}.$$

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc.) according to the following model:

$$Y_{ijklm} = \mu + S_i + C_{j(i)} + P_k + SL_l + \text{RP-MLH}_m + SL \times \text{RP-MLH}_{lm} + e_{ijklm},$$

where Y_{ijklm} = dependent variable, μ = overall mean, S_i = fixed effect of ith square, $C_{j(i)}$ = random effect of jth cow nested within ith square, P_k = fixed effect of kth period, SL_l = fixed effect of lth dietary starch level (reduced or high), RP-MLH_m = fixed effect of mth RP-MLH (with or without supplementation), SL × RP-MLH_{lm} = interaction between lth dietary starch level and mth RP-MLH, and e_{ijklm} = residual error. Normality of residuals was checked with normal probability and box plots, and homogeneity of variances was checked with plots of residual versus predicted values. Outliers were removed from statistical analyses when studentized residuals were >3.0 or <−3.0. One cow got sick and was diagnosed with displaced abomasum during period 2. A surgery was performed by the University veterinarian, and all her data from period 2 were not used in the statistical analyses. After a full recovery and outlier analyses, data from period 1 (before she got sick) and periods 3 and 4 (after surgery) were deemed adequate to be used statistically. The main effects of dietary starch level and RP-MLH supplementation and their interactions were tested using ANOVA. All animal derived results were expressed as least squares means and standard error of the mean (greatest SEM reported in Tables 5–8). Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The lack of interactions between dietary starch level and RP-MLH supplementation on DMI and milk yield and composition (Table 5), plasma concentration of most EAA and NEAA (Table 6), nutrient digestibility (Table 7), and energy balance (Table 8) showed that the effects of starch intake and RP-MLH were independent or additive. There has been limited research investigating energy by AA interactions in dairy cows supplemented with RP-AA. Rulquin and Delaby (1997) reported no interaction effects of energy intake level (87 or 100% of requirements) and RP-Met supplementation for most variables analyzed including DMI, yields of milk and milk components, and plasma concentration of AA. However, significant energy by RP-Met interactions were observed for BW gain and plasma concentration of glucose, which are discussed below.

Intake and Milk Yield and Composition

Dry matter intake, milk yield and composition, feed efficiency, BW, BCS, and concentrations of PUN and blood hemoglobin in dairy cows fed low MP diets are shown in Table 5. There were no significant interactions between dietary starch level and RP-MLH supplementation for production responses, PUN, and blood hemoglobin. A trend ($P = 0.08$) for an interaction between dietary starch level × RP-MLH supplementation was observed for milk true protein concentration. Supplementation with RP-MLH was more effective to increase milk true protein concentration in the RS than HS diet despite no interaction effect observed for milk true protein yield, suggesting no effect on milk protein synthesis.

Cows fed the HS and HS+AA diets had lower DMI (25.2 vs. 25.9 kg/d; $P = 0.02$) than those offered the RS and RS+AA diets (Table 5), possibly in response to increased propionate formation in the rumen. Accord-

ing to Allen et al. (2009), among the fuels metabolized in the liver of ruminants, propionate emerges as one of the most prominent satiety signals due to its use for gluconeogenesis and stimulation of hepatic oxidation of acetyl-CoA, resulting in the production of intermediates of the tricarboxylic acid cycle and ATP, which are all involved in satiety regulatory mechanisms. Alternatively, decreased DMI in the HS and HS+AA diets may have been caused by reduced ruminal pH, and consequent negative effects on fiber digestibility in the rumen (Allen, 1997; Brito et al., 2006; Brito and Broderick, 2006).

Despite reduced DMI, milk yield increased ($P < 0.001$) by 2.2 kg/d when cows received the HS and HS+AA diets (Table 5). It is conceivable that increased dietary starch level supplied additional glucose and glucose precursors such as propionate and lactic acid, leading to increased production of milk and milk lactose. In fact, milk lactose yield was 9% greater ($P < 0.001$) with feeding the HS and HS+AA than RS and RS+AA diets. Rulquin and Delaby (1997) observed that the plasma concentration of glucose increased only when RP-Met was supplemented to the low-energy diet (i.e., 87% of the requirements), suggesting a role of Met on gluconeogenesis. Rius et al. (2010a) also detected a significant interaction between abomasal infusions of starch and casein for the concentration of glucose in plasma of feed-restricted dairy cows (70% of ad libitum intake). Specifically, plasma glucose was greater when starch was infused in the absence of casein (Rius et al., 2010a). Increased milk yield concomitant with reduced DMI improved ($P < 0.01$) feed efficiencies (milk yield/DMI, 4% FCM yield/DMI, and ECM yield/DMI) in the HS and HS+AA versus RS and RS+AA diets (Table 5).

Boerman et al. (2015) reported that milk yield decreased by 3.9 kg/d when feeding soyhulls (30% of the diet DM) at the expense of ground corn, which agrees with present results. In contrast, previous studies revealed no changes in milk yield when substituting corn grain with incremental amounts of either soyhulls (Ipharraguerre et al., 2002a) or pelleted beet pulp (Voelker and Allen, 2003a). These discrepant responses may be explained, at least partially, by differences in

Table 5. Dry matter intake, milk yield and composition, plasma urea N (PUN), blood hemoglobin (Hb), BCS, and BW in lactating dairy cows fed low MP diets with different starch levels (SL) supplemented or not with rumen-protected Met, Lys, and His (RP-MLH[1])

	Treatment[2]					*P*-value[3]		
Item	RS	RS+AA	HS	HS+AA	SEM	SL	RP-MLH	SL × RP-MLH
DMI, kg/d	25.8	26.0	25.1	25.2	0.68	0.02	0.59	0.87
Starch intake, kg/d	2.77	2.93	7.73	7.75	0.19	<0.001	0.46	0.55
Milk yield, kg/d	37.4	38.3	40.2	40.0	1.16	<0.001	0.49	0.34
Milk yield/DMI, kg/kg	1.46	1.48	1.61	1.60	0.03	<0.001	0.71	0.60
4% FCM,[4] kg/d	37.8	37.4	37.8	38.4	1.08	0.37	0.88	0.44
4% FCM/DMI, kg/kg	1.48	1.44	1.52	1.54	0.03	<0.01	0.75	0.17
ECM,[5] kg/d	39.5	39.4	40.2	40.8	1.13	0.09	0.63	0.60
ECM/DMI, kg/kg	1.54	1.52	1.61	1.63	0.03	<0.001	0.98	0.26
Milk fat, %	4.05	3.97	3.64	3.74	0.11	<0.001	0.82	0.14
Milk fat, kg/d	1.51	1.49	1.45	1.49	0.05	0.35	0.84	0.33
Milk true protein, %	2.80	2.91	2.87	2.91	0.06	0.02	<0.001	0.08
Milk true protein, kg/d	1.05	1.09	1.15	1.17	0.03	<0.001	0.13	0.43
Milk lactose, %	4.93	4.83	4.98	4.92	0.06	0.05	0.02	0.60
Milk lactose, kg/d	1.86	1.82	2.02	1.98	0.06	<0.001	0.20	0.95
Milk N, % of N intake	25.0	24.9	28.9	28.2	0.50	<0.001	0.22	0.39
MUN, mg/dL	12.5	12.6	10.8	11.2	0.69	<0.01	0.54	0.82
PUN, mg/dL	13.2	13.4	11.3	11.9	0.54	<0.001	0.38	0.64
Blood Hb, g/dL	10.2	10.3	10.1	10.2	0.15	0.15	0.40	0.51
BCS[6]	2.83	2.91	2.90	2.87	0.11	0.74	0.63	0.29
BW, kg	703	702	703	704	12.2	0.64	0.96	0.78
BW change, kg/d	0.05	0.32	0.09	0.39	0.15	0.71	0.06	0.91

[1]RP-MLH = 25 g/d of RP-Met (Smartamine M; Adisseo USA Inc.), 76 g/d of RP-Lys (AjiPro-L; Ajinomoto Health & Nutrition North America Inc.), and 110 g/d of RP-His (prototype supplement; Ajinomoto Co. Inc.).

[2]RS (reduced starch, 12.3% starch) = diet containing 20% pelleted beet pulp and 10% soyhulls, RS+AA = RS diet supplemented with RP-MLH, HS (high starch, 34.4% starch) = diet containing 30% ground corn, and HS+AA = HS diet supplemented with RP-MLH.

[3]SL = main effect of dietary starch level, RP-MLH = main effect of RP-MLH supplementation, and SL × RP-MLH = interaction between dietary starch level and RP-MLH supplementation.

[4]4% FCM = (0.4 × kg of milk) + (15 × kg of milk fat); Gaines and Davidson (1923).

[5]ECM = 0.327 × milk yield (kg/d) + 12.95 × milk fat (kg/d) + 7.20 × milk true protein (kg/d); Tyrrell and Reid (1965).

[6]On a 1 to 5 scale, where 1 = thin and 5 = fat (Wildman et al., 1982).

milk yield and NE_L requirements in cows used across experiments.

Milk fat concentration decreased ($P < 0.001$) by 8% when NFFS were replaced with ground corn in the present study (Table 5). Contrarily, milk fat yield was similar ($P = 0.35$) and averaged 1.49 kg/d across treatments. This reduction in milk fat concentration was likely caused by a dilution effect due to an increase of 2.2 kg/d of milk yield with feeding the HS and HS+AA versus RS and RS+AA diets. However, a greater magnitude difference in milk yield between treatments (i.e., 3.9 kg/d) in the study of Boerman et al. (2015) did not affect milk fat concentration (mean = 3.81%), implying that additional factors might have been involved. Boerman et al. (2015) fed less corn silage (22.5 vs. 35.7%) and included 5% of wheat straw (DM basis) in their diets compared with the present study. Increased ruminal starch digestibility of corn grain from corn silage together with reduced supply of physically effective fiber may have lowered ruminal pH and shifted biohydrogenation pathways toward *trans*-10 18:1 and *trans*-10,*cis*-12 18:2 fatty acids, which are known to depress milk fat synthesis in the mammary gland (Baumgard et al., 2002; Shingfield et al., 2009). Alternatively, decreased ($P < 0.001$; Table 7) apparent total-tract NDF and ADF digestibilities in cows fed the HS and HS+AA diets suggested less availability of ruminal acetate for de novo synthesis of fatty acids in mammary tissues.

Cows fed the HS and HS+AA diets had greater ($P \leq 0.02$) concentrations and yields of milk true protein than those offered the RS and RS+AA diets (Table 5). Boerman et al. (2015) observed that milk protein concentration and yield were greater in dairy cows fed ground corn versus soyhulls both at 30% of the diet DM. It can be hypothesized that enhanced starch intake ($P < 0.001$; Table 5) promoted mammary uptake of EAA through improved glucose supply to support milk protein synthesis, which agrees with Rius et al. (2010a), who abomasally infused starch versus casein in feed-restricted (70% of ad libitum intake) dairy cows. In fact, the plasma concentrations of all EAA, except Met and Thr, decreased with feeding the HS and HS+AA versus RS and RS+AA diets (Table 6). Rius et al. (2010a) also showed that abomasal infusion of starch increased the plasma concentrations of insulin and IFG-I, which are known to stimulate milk protein synthesis. Blood hemoglobin concentration, BCS, BW, and BW change were not affected by dietary starch level in the present study (Table 5).

We observed that feeding the HS and HS+AA versus RS and RS+AA diets decreased ($P < 0.01$) MUN and PUN concentrations by 13% and increased ($P < 0.001$) milk N efficiency by 14%. Reduced MUN and PUN concentrations in cows fed the HS and HS+AA diets may have been a consequence of increased supply of fermentable energy from starch, resulting in NH_3-N more efficiently captured for microbial protein synthesis in the rumen than for hepatic ureagenesis despite no treatment effects in the urinary excretion of total purine derivatives (Table 7). Intake of N was 37 g/d lower ($P < 0.001$; Table 7) in cows fed the HS and HS+AA than RS and RS+AA diets, which may have also affected MUN and PUN concentrations. Fredin et al. (2015b) observed an 8% decrease in MUN concentration in cows fed normal starch (mean = 26.5%) versus reduced starch (mean = 18.2%) diets, indicating that ruminally fermentable energy from ground corn led to better N use efficiency than that from soyhulls. In contrast, PUN (O'Mara et al., 1997) and both MUN and PUN concentrations (Borucki Castro et al., 2008) were not changed when ground corn (O'Mara et al., 1997) or high-moisture corn (Borucki Castro et al., 2008) was partially replaced with beet pulp in diets of dairy cows. These discrepant results may be partly explained by the magnitude difference in starch level between treatments across different studies, as well as by the ingredient composition of the basal diets (i.e., proportion of RDP vs. RUP sources).

No significant changes in DMI, milk yield, and feed efficiencies were observed in cows offered low MP diets supplemented with RP-MLH (Table 5). Our results agree with Lee et al. (2015) and Giallongo et al. (2016), who fed MP-deficient diets supplemented with RP-ML and RP-MLH, respectively. Contrarily, Lee et al. (2012) observed that RP-MLH significantly improved milk yield in dairy cows offered a diet with greater MP deficiency (i.e., 13%) than those fed by Lee et al. (2015) and Giallongo et al. (2016), which averaged 10 and 2% MP deficiency, respectively. Even though yields of milk components were not changed by RP-MLH supplementation (Table 5), previous research showed that RP-MLH improved yields of milk fat (Giallongo et al., 2016), milk protein (Lee et al., 2012; Giallongo et al., 2016), and milk lactose (Lee et al., 2012). The lack of response in milk true protein yield with supplemental RP-MLH suggested that diets fed herein were not MP-deficient or only marginally deficient. Concentration of milk true protein increased ($P < 0.001$) in dairy cows fed the RS+AA and HS+AA diets, despite increased milk volume (Table 5). However, yield of milk true protein was not affected ($P = 0.13$), indicating no effect of RP-MLH on milk protein synthesis. Milk lactose concentration decreased ($P = 0.02$) in cows supplemented with RP-MLH, which may be explained by a dilution effect caused by improved milk volume (Table 5).

Table 6. Concentrations (μM) of plasma AA and the His-containing metabolites carnosine and 3-methylhistidine in lactating dairy cows fed low MP diets with different starch levels (SL) supplemented or not with rumen-protected Met, Lys, and His (RP-MLH[1])

	Treatment[2]					*P*-value[3]		
Item	RS	RS+AA	HS	HS+AA	SEM	SL	RP-MLH	SL × RP-MLH
EAA								
Arg	81.8	95.0	70.4	72.7	3.27	<0.001	<0.01	0.04
His	43.0	55.2	37.1	50.9	2.01	<0.01	<0.001	0.67
Ile	139	147	108	111	4.68	<0.001	0.18	0.52
Leu	132	137	117	118	5.68	<0.001	0.48	0.75
Lys	77.2	95.3	66.5	70.7	3.68	<0.001	<0.01	0.03
Met	21.1	37.5	23.2	37.5	1.55	0.40	<0.001	0.37
Phe	48.1	51.0	43.5	43.5	1.42	<0.001	0.28	0.28
Thr	96.5	104	97.5	95.8	3.93	0.24	0.33	0.14
Trp	47.9	48.8	45.7	43.7	1.27	<0.01	0.67	0.23
Val	250	262	202	205	9.09	<0.001	0.22	0.46
Total	936	1,037	810	848	28.9	<0.001	<0.01	0.18
NEAA								
Ala	275	299	298	291	15.1	0.41	0.38	0.13
Asn	44.9	51.2	44.7	44.7	1.91	0.05	0.06	0.06
Asp	2.86	3.07	3.16	3.18	0.13	0.06	0.29	0.37
Cit	88.6	95.0	89.7	87.3	4.22	0.20	0.44	0.09
Cys	15.9	17.8	18.0	18.9	0.56	<0.001	<0.001	0.11
Gln	210	220	206	208	6.87	0.21	0.32	0.51
Glu	38.7	39.0	39.6	40.3	1.72	0.27	0.63	0.85
Gly	242	245	299	285	9.90	<0.001	0.46	0.29
Orn	39.4	47.5	36.5	37.8	2.38	<0.001	<0.01	0.03
Pro	81.2	88.2	92.5	90.0	4.44	0.03	0.44	0.11
Ser	71.2	74.5	77.3	74.3	2.56	0.14	0.95	0.11
Taurine	30.6	40.1	39.0	45.6	1.86	<0.001	<0.001	0.33
Tyr	49.3	52.2	46.2	45.2	2.57	<0.01	0.59	0.30
Total	1,191	1,272	1,292	1,274	35.9	0.06	0.25	0.07
Carnosine	17.4	18.0	18.2	18.7	0.66	0.19	0.37	0.97
3-Methylhistidine	1.58	1.30	1.60	1.41	0.11	0.55	0.04	0.71

[1]RP-MLH = 25 g/d of RP-Met (Smartamine M; Adisseo USA Inc.), 76 g/d of RP-Lys (AjiPro-L; Ajinomoto Health & Nutrition North America Inc.), and 110 g/d of RP-His (prototype supplement; Ajinomoto Co. Inc.)

[2]RS (reduced starch, 12.3% starch) = diet containing 20% pelleted beet pulp and 10% soyhulls, RS+AA = RS diet supplemented with RP-MLH, HS (high starch, 34.4% starch) = diet containing 30% ground corn, and HS+AA = HS diet supplemented with RP-MLH.

[3]SL = main effect of dietary starch level, RP-MLH = main effect of RP-MLH supplementation, and SL × RP-MLH = interaction between dietary starch level and RP-MLH supplementation.

A trend ($P = 0.06$) for increased BW gain was observed in cows supplemented with RP-MLH, but RP-MLH did not affect ($P \geq 0.38$) final BW, BCS, and concentrations of MUN, PUN, and blood hemoglobin (Table 5). Rulquin and Delaby (1997) reported a significant interaction between dietary energy level and RP-Met supplementation for final BW in lactating dairy cows. Specifically, RP-Met either decreased (low energy diet) or increased (normal energy diet) the final BW by 4 kg when supplemented to lactating dairy cows (Rulquin and Delaby, 1997).

Plasma AA and His-containing Metabolites

The plasma concentrations of EAA, NEAA, and the His-containing metabolites carnosine and 3-methylhistidine (**3-MHis**) in dairy cows fed low MP diets are shown in Table 6. Significant interaction effects between dietary starch level and RP-MLH supplementation were observed for plasma Arg and Lys. Although RP-MLH supplementation to the RS diet increased ($P < 0.001$) the plasma concentrations of Arg and Lys by 16 and 23%, respectively, no changes were observed when RP-MLH was supplemented to the HS diet. These interactions may have been caused by less mammary extraction of Arg and Lys with feeding NFFS due to decreased starch intake and yields of milk and milk true protein (Table 5). It should be noted that infusion of Arg via the jugular vein or abomasum (Vicini et al., 1988) or its deletion from a mixture of AA infused postruminally (Schwab et al., 1976; Doepel and Lapierre, 2010) had no effect on milk protein yield.

Rulquin and Delaby (1997) observed no significant interaction between energy intake and RP-Met supplementation on the plasma concentrations of EAA, which generally agree with our results. Likewise, Rius et al. (2010a) found no significant casein by starch interactions for the plasma concentrations of most EAA apart

from Leu and Trp, which increased in response to abomasal infusion of casein in the absence of starch in feed-restricted dairy cows. In addition, Rius et al. (2010a) reported significant interactions for the mammary clearance rates of several EAA including His, Met, Phe, and Thr, and trends for Lys and Trp, with casein increasing clearance rates of these AA when infused alone and decreasing when infused together with starch. According to Rius et al. (2010a), the supply of EAA used to improve milk protein synthesis during starch infusion was facilitated by increased plasma flow to mammary tissues and increased clearance rates of most EAA, resulting in a net uptake of AA by the mammary gland. Therefore, decreased plasma concentrations of Arg and Lys when RP-MLH was supplemented to the RS diet but not to the HS counterpart (Table 6) may be linked to increased starch supply even though the mechanism or mechanisms behind selective extraction of certain AA by mammary cells have yet to be fully understood.

Compared with cows fed the RS and RS+AA diets, those receiving the HS and HS+AA diets had lower ($P < 0.01$) plasma concentrations of all EAA, except Met and Thr, which were not affected ($P \geq 0.17$) by dietary starch level (Table 6). Enhanced starch intake with feeding the HS and HS+AA diets led to an 8% increase in milk true protein yield potentially caused increased extraction of AA by mammary tissues, which is in line with the observed reduction in the plasma concentration of most EAA. Rius et al. (2010a) showed that abomasal infusions of starch and starch plus casein resulted in lower plasma concentrations of Ile, His, Lys, Phe, and Val than infusions of water and casein in feed-restricted dairy cows. They also reported increased mammary plasma flow and clearance rates of Arg, Ile, Leu, Lys, and Trp (Rius et al., 2010a). Alternatively, the decline in plasma concentrations of EAA in the HS and HS+AA diets may be explained by decreased AA catabolism as suggested by Rius et al. (2010a) and corroborated by the reductions in MUN and PUN concentrations when replacing NFFS with ground corn (Table 5). In addition, decreased MUN and PUN in cows fed HS and HS+AA diets was accompanied by increased milk N efficiency (Table 5) and lowered urinary excretion of urea N (Table 7), which together indicate less catabolic losses of AA and improved postabsorptive efficiency (Rius et al., 2010a).

A significant dietary starch level × RP-MLH interaction effect was detected for the plasma concentration of Orn, which increased by 21% when the RS diet was

Table 7. Apparent total-tract digestibility of nutrients and urinary excretion of nitrogenous compounds in lactating dairy cows fed low MP diets with different starch levels (SL) supplemented or not with rumen-protected Met, Lys, and His (RP-MLH[1])

	Treatment[2]					*P*-value[3]		
Item	RS	RS+AA	HS	HS+AA	SEM	SL	RP-MLH	SL × RP-MLH
Apparent total-tract digestibility, %								
DM	71.0	70.4	70.9	70.6	0.36	0.85	0.19	0.67
OM	72.3	72.2	72.2	71.6	0.42	0.36	0.38	0.57
CP	69.2	68.0	69.0	68.6	0.59	0.66	0.07	0.30
NDF	59.7	59.2	44.9	43.9	0.74	<0.001	0.27	0.75
ADF	63.4	62.2	48.8	47.7	0.92	<0.001	0.11	0.93
Starch	97.9	98.2	99.1	99.1	0.21	<0.001	0.46	0.52
Ether extract	85.8	82.6	87.3	84.6	0.49	<0.001	<0.001	0.61
N intake, g/d	677	704	642	666	17.5	<0.001	<0.01	0.83
Urinary excretion								
Creatinine, m*M*	4.71	5.01	5.65	5.11	0.46	0.16	0.74	0.25
Volume, L/d	39.0	37.6	36.3	37.2	2.25	0.36	0.90	0.48
Urea N, g/d	196	195	149	168	9.88	<0.001	0.34	0.29
Total N, g/d	323	325	297	317	14.0	0.17	0.37	0.44
Urea N, % of total N	63.2	59.6	50.7	54.8	2.36	<0.001	0.89	0.06
Urea N, % N intake	29.3	28.1	23.5	25.9	1.76	0.02	0.71	0.24
Total N, % N intake	47.4	46.7	46.8	48.0	1.95	0.83	0.90	0.60
Uric acid, mmol/d	102	102	93.5	91.9	5.38	0.06	0.85	0.90
Allantoin, mmol/d	596	584	575	620	36.5	0.80	0.56	0.32
Total PD,[4] mmol/d	699	684	665	704	39.4	0.83	0.71	0.39

[1]RP-MLH = 25 g/d of RP-Met (Smartamine M; Adisseo USA Inc.), 76 g/d of RP-Lys (AjiPro-L; Ajinomoto Health & Nutrition North America Inc.), and 110 g/d of RP-His (prototype supplement; Ajinomoto Co. Inc.).

[2]RS (reduced starch, 12.3% starch) = diet containing 20% pelleted beet pulp and 10% soyhulls, RS+AA = RS diet supplemented with RP-MLH, HS (high starch, 34.4% starch) = diet containing 30% ground corn, and HS+AA = HS diet supplemented with RP-MLH.

[3]SL = main effect of dietary starch level, RP-MLH = main effect of RP-MLH supplementation, and SL × RP-MLH = interaction between dietary starch level and RP-MLH supplementation.

[4]PD = purine derivatives (uric acid + allantoin).

Table 8. Emissions[1] of CO_2 and CH_4, dietary energy parameters, and milk energy efficiencies in lactating dairy cows fed low MP diets with different starch level (SL) supplemented or not with rumen-protected Met, Lys, and His (RP-MLH[2])

	Treatment[3]					*P*-value[4]		
Item	RS	RS+AA	HS	HS+AA	SEM	SL	RP-MLH	SL × RP-MLH
Gaseous emissions								
CO_2, kg/d	11.8	12.0	12.1	11.9	0.32	0.79	0.99	0.46
CH_4, g/d	545	545	434	434	20.8	<0.001	0.98	0.99
CH_4, g/kg of DMI	21.2	21.1	17.2	17.4	0.78	<0.001	0.96	0.77
CH_4, g/kg of ECM	13.5	13.7	10.7	10.6	0.47	<0.001	0.88	0.65
Fractions,[5] Mcal/d								
GE intake	107	108	107	107	2.82	0.42	0.58	0.85
DE intake	78.9	78.3	76.7	76.8	1.87	0.03	0.77	0.66
ME intake	67.3	66.3	66.6	66.5	1.83	0.76	0.53	0.57
NE_L intake	46.2	45.3	45.6	45.7	1.53	0.94	0.63	0.49
Components,[6] Mcal/d								
Fecal energy	28.6	30.1	30.0	30.3	1.07	0.27	0.17	0.39
Urinary energy	4.72	4.74	4.33	4.63	0.20	0.17	0.36	0.44
CH_4 energy	7.19	7.18	5.72	5.71	0.28	<0.001	0.97	0.99
Heat production	30.1	30.4	30.6	30.2	0.79	0.76	1.00	0.48
Milk energy	27.3	27.1	27.8	28.2	0.80	0.08	0.80	0.58
Tissue energy	7.62	6.90	6.59	6.33	1.09	0.25	0.48	0.74
Efficiencies, %								
ME/DE	85.0	84.6	87.0	86.5	0.43	<0.001	0.24	0.79
Milk energy/ME	41.5	41.6	42.1	43.1	0.74	0.08	0.42	0.44
Heat production/ME	44.9	45.4	45.5	45.5	1.36	0.70	0.76	0.81
NE_L/ME	68.8	68.2	68.4	68.5	0.85	0.90	0.73	0.54

[1]Gases were measured using the GreenFeed system (C-Lock Technology Inc.). Data were derived from 8 individual spot measurements over a 4-d period.

[2]RP-MLH = 25 g/d of RP-Met (Smartamine M; Adisseo USA Inc.), 76 g/d of RP-Lys (AjiPro-L; Ajinomoto Health & Nutrition North America Inc.), and 110 g/d of RP-His (a prototype supplement; Ajinomoto Co. Inc.).

[3]RS (reduced starch, 12.3% starch) = diet containing 20% pelleted beet pulp and 10% soyhulls, RS+AA = RS diet supplemented with RP-MLH, HS (high starch, 34.4% starch) = diet containing 30% ground corn, and HS+AA = HS diet supplemented with RP-MLH.

[4]SL = main effect of dietary starch level, RP-MLH = main effect of RP-MLH supplementation, and SL × RP-MLH = interaction between dietary starch level and RP-MLH supplementation.

[5]GE intake = gross energy intake; digestible energy (DE) intake = GE intake − fecal energy; ME intake = DE intake − urinary energy − CH_4 energy; $NE_L = 0.080 \times BW^{0.75}$ + milk energy + tissue energy (NRC, 2001).

[6]Urinary energy (Mcal/d) = [14.6 × urinary N output (g/d)]/1,000 (Morris et al., 2021); CH_4 energy (Mcal/d) = [CH_4 (L/d) × 9.45 (kcal/L)]/1,000; heat production (Mcal/d) = [0.0185 × CO_2 (L/d) + 6.8]/4.184 (Bayat et al., 2019); milk energy = [(0.0929 × milk fat %) + (0.0563 × milk true protein %) + (0.0395 × milk lactose %)] × milk yield (kg/d) (NRC, 2001); tissue energy = positive tissue energy × k_G/k_T or negative tissue energy × k_T, where tissue energy = ME intake − heat production − milk energy; efficiency coefficient values of 0.70 and 0.89 were used for k_G (efficiency of utilizing ME intake for tissue gain) and k_T (efficiency of utilizing body reserve energy for milk yield), respectively (Moraes et al., 2015).

supplemented with RP-MLH, but no change was seen with RP-MLH supplementation to the HS diet (Table 6). Interaction trends ($P \leq 0.09$) were also seen for the plasma concentrations of Asn and Cit, with both following the same pattern observed for Orn. We also observed that replacing NFFS with ground corn increased ($P \leq 0.03$) the plasma concentrations of Cys, Gly, Pro, and taurine, and decreased ($P \leq 0.05$) those of Asn and Tyr (Table 6). Rius et al. (2010a) reported significant casein by starch interactions for the plasma concentrations of Ala, Cys, and Gln, with reduced Cys and increased Ala and Gln in response to abomasal infusion of casein in the presence of starch. In contrast, Rulquin and Delaby (1997) did not find a significant energy by RP-Met interaction for the plasma concentration of any individual EAA investigated. The interactions and changes in the plasma concentrations of several NEAA observed in our study possibly reflect their anabolic and catabolic use at the tissue level in association with varying supply of starch as reported in the literature (Rius et al., 2010a; Omphalius et al., 2019).

Supplementation with RP-MLH increased ($P < 0.01$) the plasma concentrations of Met, Lys, and His (Table 6), suggesting effective digestible supply of these EAA in agreement with Lee et al. (2012) and Giallongo et al. (2016). We also observed that the concentrations of plasma Cys and taurine increased in response to supplemental RP-MLH, which may be associated with the synthesis of Cys via transsulfuration of Met, and Cys being used as a precursor for taurine production (Baker, 2006). Contrarily, Lee et al. (2012) and Giallongo et al. (2016) observed no changes in plasma taurine concentration in dairy cows supplemented with RP-MLH, probably because Met was prioritized

for milk protein synthesis as dairy cows were in more negative MP balance compared with those used herein. All remaining concentrations of individual EAA and NEAA and carnosine in plasma were not significantly affected by RP-MLH supplementation in the present study (Table 6).

Although dietary starch level did not affect the plasma concentrations of carnosine ($P = 0.19$) and 3-MHis ($P = 0.55$), RP-MLH supplementation decreased ($P = 0.04$) the concentration of 3-MHis in plasma (Table 6). According to Houweling et al. (2012), the catabolism of actin and myosin releases 3-MHis from skeletal muscles, with 3-MHis being considered a reliable indicator of muscle proteolysis in cattle (Harris and Milne, 1981). Body weight gain tended ($P = 0.06$; Table 5) to increase in dairy cows fed the RS+AA and HS+AA versus RS + HS diets, suggesting tissue accretion rather than mobilization, thus in line with decreased 3-MHis. Note that the concentration of 3-MHis averaged 1.47 μM across diets (Table 6) and was 57% lower than that reported by Giallongo et al. (2015) and Zang et al. (2019), which averaged 3.52 and 3.32 μM, respectively. This discrepancy in plasma 3-MHis concentration among these experiments cannot be attributed to variation in MP balances. While the MP balances of the basal diets fed in Giallongo et al. (2015) and herein were remarkably similar (−145 g/d), that from the basal diet used by Zang et al. (2019) was less negative and averaged −29 g/d. Blum et al. (1985) reported no diurnal and postprandial changes in the plasma concentration of 3-MHis in crossbred and purebred lactating dairy cows during a 24-h period with frequent jugular blood sampling. Therefore, the differences in blood sampling frequency (1 vs. 3 times) and sampling time (1000 h vs. 1300, 1700, and 2100 h) between the present study and Giallongo et al. (2015) do not appear to account for the observed discrepancy in 3-MHis concentration in plasma. However, Ndibualonji et al. (1997) observed that the plasma concentration of 3-MHis obtained every 10-min interval via the jugular vein was lower during the morning (0600–1200 h; mean = 9.2 μM) than afternoon (1600–2200 h; mean = 10.6 μM) postprandial period in nonpregnant, nonlactating dairy cows submitted to short-term feed deprivation.

Nutrient Digestibility and Urinary N Excretion

Apparent total-tract digestibilities of nutrients and urinary excretion of nitrogenous metabolites in dairy cows fed low MP diets are presented in Table 7. No significant dietary starch level by RP-MLH supplementation interaction effects were observed for the apparent total-tract digestibility of nutrients and the urinary excretion of nitrogenous compounds. Dietary starch level did not change ($P \geq 0.36$) the apparent total-tract digestibilities of DM, OM, and CP, which agrees with Ipharraguerre et al. (2002b). However, the apparent total-tract digestibilities of NDF and ADF decreased ($P < 0.001$) by 25 and 23%, respectively, in cows fed the HS and HS+AA versus RS and RS+AA diets, thus in line with previous results (Voelker and Allen, 2003b; Boerman et al., 2015; Fredin et al., 2015a). According to Ipharraguerre and Clark (2003), dietary NDF concentration and NDF total-tract digestibility increase when soyhulls replace corn grain in dairy diets because of the low lignin content of soyhulls-NDF. Despite reduced total-tract digestibilities of NDF and ADF with feeding the HS and HS+AA diets, DM and OM digestibilities were unaffected, implying that starch may have offset losses in fiber digestibility.

Feeding the HS and HS+AA diets increased the apparent total-tract digestibility of starch by 1 percentage unit compared with the RS and RS+AA counterparts (Table 7). Contrarily, Boerman et al. (2015) reported no treatment effect in the apparent total-tract digestibility of starch with feeding ground corn versus soyhulls. While Voelker and Allen (2003b) showed that increasing levels of pelleted beet pulp at the expense of high-moisture corn did not affect the apparent total-tract digestibility of starch, Ipharraguerre et al. (2002b) observed a quadratic decrease in the total-tract digestibility of NSC when replacing ground corn with varying amounts of soyhulls. These discrepant responses across experiments may be explained by differences in DMI and production levels, and whether changes in ruminal starch digestibility (increase or decrease) are offset by starch digestibility in the lower tract.

Supplementation of RP-MLH had no effect on the apparent total-tract digestibility of nutrients apart from ether extract that decreased ($P < 0.001$) from 86.7 to 83.6% in cows fed the RS and HS versus RS+AA and HS+AA diets (Table 7). This reduction in ether extract digestibility may have been associated with increased fecal output of encapsulated lipids from undigested RP-AA supplements. Lee et al. (2012) reported that the apparent total-tract digestibilities of DM and OM decreased in lactating dairy cows fed a MP-deficient basal diet supplemented with RP-MLH compared with the same basal diet without RP-MLH supplementation. However, these differences in DM and OM digestibilities were small, ranging from 1.2 to 1.4 percentage units, and they cannot be explained by NDF and ADF digestibilities, which did not change with supplementing RP-MLH to the MP-deficient basal diet (Lee et al., 2012).

Feeding the HS and HS+AA diets reduced ($P \leq 0.02$) the urinary excretion of urea N (g/d, % of total urinary N, and % of N intake) compared with the RS

and RS+AA diets (Table 7), which may have resulted from less circulating NH_3 being used for hepatic ureagenesis. In fact, reduced concentrations of MUN and PUN together with improved milk N efficiency in cows fed the HS and HS+AA diets (Table 5) support this hypothesis. In contrast, the urinary excretion of total N, expressed as a proportion of N intake, did not differ significantly across treatments (Table 7). It should be noted that, on average, 47% of the N intake was excreted as N in urine, suggesting that urinary volume was likely overestimated. Lee et al. (2019) showed that compared with total collection of urine, spot sampling (12 time points) underestimated urinary volume by 8.6%. They also concluded that a sampling frequency of at least 6 equally-spaced time interval spot samples in a 24-h cycle was required for obtaining reliable comparisons of urinary outputs between diets (Lee et al., 2019). The overestimation of urinary volume in our study occurred despite intensive urinary sampling (8 time points), implying bias due to large individual cow variation in the excretion of creatinine per unit of BW (Tebbe and Weiss, 2018; Lee et al., 2019). On average, our urinary volume was 10.7 kg/d greater than that reported by Lee et al. (2019), which was obtained via total urine collection from cows also fed a high corn silage diet (i.e., 48.5%, DM basis). Potassium intake, which has been shown to increase urinary volume in a dose-response fashion (Eriksson and Rustas, 2014), was similar comparing the corn silage–based diet (mean = 333 g/d) fed by Lee et al. (2019) with that used in our experiment (mean = 290 g/d; data not shown). Therefore, dietary concentration of potassium does not explain the observed overestimation in urinary volume. The mean urinary creatine concentration and urinary volume in the present study were 23% lower (5.12 vs. 6.67 m*M*) and 26% greater (37.5 vs. 29.8 kg/d) than those reported by Lee et al. (2019). These discrepancies occurred despite using the same creatinine analytical method and excretion rate coefficient (i.e., 29 mg/kg of BW) in both studies, but less sampling frequency in our experiment (8 vs. 12 spot urine samples). Using smaller excretion rate coefficients for creatinine from studies that performed total collection of urine [24.1 mg/kg of BW (Chizzotti et al., 2008) and 27.3 mg/kg of BW (Lee et al., 2019)] would result in mean urinary volumes of 31.8 and 36 kg/d and urinary N excretions of 274 and 310 g/d.

Neither dietary starch level nor RP-MLH supplementation affected the urinary excretion of total purine derivatives (Table 7). Supplementation with RP-MLH also had no significant effect in the urinary excretion of urea N and total N expressed in grams per day or as a proportion of N intake. Feeding the HS and HS+AA versus RS and RS+AA diets tended ($P = 0.06$) to decrease the urinary excretion of uric acid (Table 7). This reduction in uric acid did not affect total purine derivatives excretion, suggesting no effect of diets on microbial protein synthesis in the rumen. However, previous research demonstrated that urinary purine derivatives was less precise and accurate as a microbial marker to detect differences between treatments in microbial protein synthesis than ^{15}N (Reynal et al., 2005).

Gaseous Emissions and Energy Utilization

Emissions of CO_2 and CH_4, and energy utilization components in dairy cows fed low MP diets are presented in Table 8. No significant interaction effects between dietary starch level and RP-MLH supplementation were observed for gaseous emissions and energy utilization variables. Feeding the HS and HS+AA diets reduced ($P < 0.001$) enteric CH_4 production, CH_4 yield, and CH_4 intensity by 20, 18, and 21%, respectively. These reductions in CH_4 emissions were possibly associated with decreased NDF intake, which was 26% lower in dairy cows fed the HS and HS+AA versus the RS and RS+AA diets (data not shown). Nielsen et al. (2013) and Niu et al. (2018) reported positive relationships between CH_4 emissions and dietary NDF concentration. Replacing NFFS with ground corn did not affect ($P = 0.79$) CO_2 emissions (Table 8).

Feeding the HS and HS+AA versus RS and RS+AA diets did not affect ($P \geq 0.42$) intake of GE, ME, and NE_L (Table 8). In contrast, intake of DE decreased ($P = 0.03$) by 1.8 Mcal/d in dairy cows fed the HS and HS+AA diets. However, this reduction in DE intake was not accompanied by dietary effects on GE intake ($P = 0.42$) and fecal energy excretion ($P = 0.27$), indicating negligible biological relevance. Likewise, dietary starch level did not affect ($P \geq 0.17$) urinary energy, heat production, and tissue energy. Methane energy and milk energy outputs followed CH_4 production (Table 7) and milk yield (Table 5) and either decreased ($P < 0.001$) or tended ($P = 0.08$) to increase, respectively, with feeding the HS and HS+AA versus RS and RS+AA diets. Energy efficiencies, expressed as ME/DE ($P < 0.001$) and milk energy/ME ($P = 0.08$), were greater in cows fed the HS and HS+AA than RS and RS+AA diets (Table 8). These efficiency responses together with increased milk energy and reduced CH_4 energy losses indicate improved energy utilization with replacing ground corn for NFFS.

Supplementation of low MP diets with RP-MLH did not affect ($P \geq 0.17$) energy utilization variables in the present study (Table 8). These results were not surprising as supplemental RP-MLH had no effects on DMI, milk yield, concentrations (except milk true protein) and yields of milk components, and CH_4 production.

CONCLUSIONS

This study was designed to test the hypothesis that different dietary starch level, achieved by replacing pelleted beet pulp and soyhulls with ground corn, and RP-MLH supplementation could interact to modulate production and nutrient utilization in dairy cows fed low MP diets. However, significant interactions were observed only for the plasma concentrations of Arg, Lys, and Orn, with these AA increasing in response to RP-MLH when supplemented to the RS but not to the HS diet. Therefore, the effects of dietary starch concentration and RP-MLH supplementation on DMI, yields of milk and milk components, plasma concentration of most EAA, and N and energy utilization were independent or additive. Increased starch intake improved yields of milk and milk true protein and milk N efficiency, and reduced urinary urea N excretion and CH_4 emissions, but RP-MLH supplementation had limited effects on lactation performance and nutrient utilization under the conditions of the present study.

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