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Histidine dose-response effects on lactational performance and plasma amino acid concentrations in lactating dairy cows: 2. Metabolizable protein-deficient diet

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ABSTRACT

The objective of this experiment was to determine the effect of increasing digestible His (dHis) levels with a rumen-protected (RP) His product on milk production, milk composition, and plasma AA concentrations in lactating dairy cows fed a metabolizable protein (MP)-deficient diet, according to the National Research Council dairy model from 2001. The companion paper presents results on the effect of increasing dHis dose with a MP-adequate basal diet. Twenty Holstein cows, of which 8 were rumen-cannulated, were used in a replicated 4 × 4 Latin square design experiment with four 28-d periods. Treatments were a control diet supplying 1.8% dHis of MP or 37 g/d (dHis1.8) and the control diet supplemented RP-His to provide 2.2, 2.6, or 3.0%, dHis of MP, or 53, 63, and 74 g/d (dHis2.2, dHis2.6, and dHis3.0, respectively). Histidine dose did not affect dry matter intake, but milk yield increased quadratically and energy-corrected milk yield increased linearly with increasing dHis dose. Histidine dose had a quadratic effect on milk fat concentration but did not affect milk fat yield. Lactose concentration decreased linearly, whereas lactose yield increased linearly with increasing dHis dose. There was a tendency for a linear increase in milk true protein concentration, and milk true protein yield increased linearly with dHis dose. Further, plasma His concentration increased linearly with increasing dHis dose and calculated apparent efficiency of His utilization decreased quadratically with increasing dHis supply. Histidine had minor or no effects on rumen fermentation. In the conditions of this experiment, RP-His supplementation of an MP-deficient corn silage-based diet increased milk yield linearly up to a dHis supply of 63 g/d (or 2.6% dHis of MP) and increased feed efficiency, energy-corrected milk yield and milk true protein yield linearly up to a dHis supply of 74 g/d (or 3.0% dHis of MP) in lactating dairy cows.

Key words: histidine, milk production, milk protein, dairy cow

INTRODUCTION

Ruminants are relatively poor utilizers of dietary true protein: in dairy cattle N efficiency (% of dietary N incorporated into milk N) varies between 14 and 45%, with an average of 25% (Huhtanen and Hristov, 2009). Lowering the protein content of the diet has been consistently shown to increase N efficiency of lactating cows. Indeed, several experiments with low-CP diets (14 vs. 16% CP) have shown around 38 and 48% reduction in excretion of urinary total and urea N (Lee et al., 2012a,b, 2015). However, when the dietary supply of RUP is decreased, the cow becomes more reliant on microbial protein to supply digestible AA (**dAA**) to support milk and milk protein production. Even though rumen microbes supply all EAA (Virtanen, 1966), some can become limiting for milk production in high-producing cows (Patton et al., 2014). In typical US diets based on corn silage and soybean products, Lys and Met are considered the first 2 limiting AA (NRC, 2001), whereas His was suggested to be another potential limiting AA in situations where dietary His supply is low or microbial protein is the predominant source of dAA, or both, for the cow (Kim et al., 1999; Vanhatalo et al., 1999; Lee et al., 2012b). More recent data further suggest that Met, His, and Lys are limiting in low-protein diets, whereas Met is the most limiting in high-protein diets (Omphalius et al., 2019).

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Indeed, several experiments with MP-deficient corn silage-based diets supplemented with rumen-protected (**RP**)-His have shown a positive response in DMI, milk yield (**MY**), or milk protein production (Lee et al., 2012a; Giallongo et al., 2015; Zang et al., 2019). Similarly, lactating cows fed grass silage-based diets, which supply less RUP and are thereby more reliant on microbial protein, have shown linear responses in MY and milk protein production to postruminal supply of increasing levels of dHis (Huhtanen et al., 2002; Kim et al., 2001).

Low dietary supply of dHis has resulted in a sharp decrease in plasma His concentrations in situations where dietary MP supply was deficient (according to NRC, 2001; Lee et al., 2012a; Ouellet et al., 2014; Giallongo et al., 2016). Infusion of His postruminally in short-term experiments has consistently shown a linear increase in plasma His concentration (Huhtanen et al., 2002; Ouellet et al., 2014; Lapierre et al., 2020). Supplementation of RP-His to MP-deficient diets have also shown increased plasma His concentration in most cases (Lee et al., 2012a; Giallongo et al., 2016) and a linear increase with incremental levels of RP-His (Zang et al., 2019). However, in the study by Giallongo et al. (2015), plasma His concentration did not decrease when dHis supply was decreased from 67 g/d (MP-adequate diet) to 59 g/d (MP-deficient diet). In that study, plasma His concentration remained unchanged when 11 g/d of dHis was supplied from RP-His. The lack of decrease in plasma His concentration in some situations has been attributed to His released from endogenous His sources, mainly carnosine, 1-methylhistidine (**MH**), 3-MH, and hemoglobin (Ouellet et al., 2014; Giallongo et al., 2015; Lapierre et al., 2020), and can, to some extent, mask dietary His deficiency (Lapierre et al., 2014, 2020). Even though the His supply from these pools is not large on a gram-per-day basis, they can provide enough His to synthesize a notable amount of milk protein, as discussed in Lapierre et al. (2020).

The current experiment is 1 of 2 crossover experiments designed to test the production responses of lactating dairy cows to incremental levels of dHis supplementation to MP-adequate and MP-deficient basal diets. Results from the first experiment with a MP-adequate diet are reported in the companion paper (Räisänen et al., 2021). The objective of the current experiment was to assess the effect of increasing dietary dHis supplementation on lactational performance and plasma His concentration of lactating cows fed a MP-deficient diet, according to NRC (2001). We hypothesize that MP deficiency amplifies His deficiency and increasing levels of dietary dHis increase DMI, MY, milk protein yield, and plasma His levels linearly.

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

The animals involved in this experiment were cared for according to the guidelines of the institutional animal care and use committee at The Pennsylvania State University. The committee approved all procedures involving animals carried out in the experiment.

A total of 20 lactating Holstein cows (8 primiparous and 12 multiparous, 8 of which were fitted with 10-cm rumen cannulas; Bar Diamond Inc.) were used in the study. Cows were 64 ± 12 DIM, 584 ± 61 kg of BW, and 43.5 ± 12.4 kg of MY at the beginning of the experiment. The cows were assigned to 5 blocks based on parity, DIM, and MY. The experiment was a replicated 4 × 4 Latin square design with 4 experimental periods of 28 d each (3 wk for adaptation to treatments and 1 wk for sampling). The experiment was conducted in the tie-stall barn of the Dairy Research and Teaching Center of The Pennsylvania State University. The barn was equipped with forced-air ventilation, individual rubber mats, and water bowls allowing free access to water. Cows were milked twice daily, at 0630 and 1900 h. Before morning milking, the cows had access to an exercise area for 1 h. Feeding was done once daily at around 0800 h.

Diet and Treatments

The 4 treatments in the study were designed to supply dHis at (1) 1.80 (**dHis1.8**), (2) 2.2 (**dHis2.2**), (3) 2.6 (**dHis2.6**), and (4) 3.0% (**dHis3.0**) of MP (estimated using NRC, 2001 and with actual AA composition of the feeds; see below for details on the AA analysis). One basal diet (Table 1) was formulated to be around 85 to 90% deficient in MP and to provide adequate NE_L based on NRC (2001) recommendations for a lactating Holstein cow with an average BW of 600 kg, DMI of 25 kg/d, MY of 40.0 kg/d, milk fat of 3.84%, and milk protein of 3.00%. The basal TMR was formulated to supply 1.8% dHis of MP, or around 37 g dHis/d and was fed to the control group (dHis1.8). For treatments dHis2.2, dHis2.6, and dHis3.0, TMR was top-dressed with RP-His to provide an additional 16, 26, and 37 g/d dHis, respectively (Table 2). The amount of dHis supplied from the RP-His product was based on manufacturer's specifications (Ajinomoto Inc.). The experimental RP-His contained 44% CP, 91% His of CP and 45% fatty acids (on a DM basis). Based on manufacturer specifications, rumen escape fraction and intestinal digestibility of His were 90 and 62%, respectively. All diets were supplemented with RP-Met

(Mepron, Evonik Nutrition and Care GmbH) and RP-Lys (AjiPro-L, Ajinomoto Co. Inc.) to meet or exceed the recommendations for dLys and dMet; that is, 6.6 and 2.2% of MP, respectively (NRC, 2001; Schwab et al., 2005). Feed intake was recorded daily, and feeding rate was adjusted to yield refusals equal to 5 to 10% of the feed consumed.

Sample Collection and Measurements

Feed and TMR Sampling. Feed and TMR sampling were conducted as described in Räisänen et al. (2021). Briefly, weekly composites of the TMR, refusals, and forages were prepared from samples collected twice weekly and kept at −20°C. Concentrate feeds were sampled weekly, and composite samples were analyzed at the end of the experiment. All samples were dried at 55°C for 72 h to a constant weight for DM determination and ground using a Wiley Mill (Thomas Scientific) through a 1-mm screen. One composite sample (on equal weight basis) for each period (TMR) or the entire experiment (individual feed ingredients) was prepared for further analysis. Composited individual feed ingredient samples were sent to Cumberland Valley Analytical Services (Waynesboro, PA) for wet chemistry analysis of OM, NDF (Van Soest et al., 1991), ADF (method 973.18; AOAC International, 2000), CP (method 990.03; AOAC International, 2000), ether extract (method 954.02; AOAC International, 2000), ash (method 942.05; AOAC International, 2000) and Ca and P (method 985.01; AOAC International, 2000). Starch was analyzed as described in Hall (2009). Individual feed ingredients were analyzed for AA at the University of Missouri–Columbia's Agricultural Experiment Station Chemical Laboratories (Columbia, MO) following the procedures of Deyl et al. (1986) and Fekkes (1996). Nutrient composition of the TMR was reconstituted from analyzed values of individual feeds and their inclusion in the TMR (Table 1).

Milk Sampling. Milk yield and BW of the cows were recorded daily at each milking. Milk samples from 2 consecutive p.m. and a.m. milkings were collected during experimental wk 4 in 20-mL tubes, preserved with 2-bromo-2-nitropropane-1,3-diol and analyzed for fat, true protein, MUN, and lactose by Dairy One Laboratory (Ithaca, NY).

Fecal and Urine Sampling. Eight fecal and urine samples each were collected every 6 h over the last 3 d of each period (at 0500, 1100, 1700, and 2300 h on d 1; at 0800, 1400, and 2000 h on d 2; and at 0200 h on d 3). Fecal samples were processed and analyzed for N, ADF, NDF, starch and indigestible NDF, and urine samples were processed and analyzed for allantoin, uric acid, urea N (**UUN**), total N, and creatinine (urine samples) as described in Räisänen et al. (2021). Composited TMR and fecal samples for each experimental period and cow were incubated for 12 d in the rumen for analysis of indigestible NDF (Huhtanen et al., 1994; Lee et al., 2012a), which was used to calculate apparent and total-tract digestibility of dietary nutrients (Schneider and Flatt, 1975). Daily urinary volume was estimated based on urinary creatinine concentration, assuming a creatinine excretion rate of 29 mg/kg of BW (Hristov

Table 1. Ingredient and chemical composition of the basal diet used in the experiment

Composition	% of DM
Feed ingredients[1]	
Corn silage[2]	45.7
Alfalfa haylage[3]	15.6
Straw/hay mix[4]	3.96
Ground corn	15.6
Roasted soybeans	1.98
Optigen	0.45
Feather meal[5]	3.76
Whole cottonseed	4.47
Molasses	4.99
Mineral mix[6]	2.89
Mepron	0.18
AjiPro-L	0.57
Chemical composition[7]	
CP	15.9
NDF	31.3
ADF	20.0
NFC	45.5
Starch	31.4
NE_L,[8] Mcal/kg	1.54
Ash	4.31
Ca	0.80
P	0.31

[1]Optigen = slow-release urea (Alltech Inc.); molasses (Westway Feed Products); Mepron (Evonik Nutrition and Care GmbH); AjiPro-L (Ajinomoto Co. Inc.).

[2]Corn silage was 42.0% DM and contained (DM basis) 7.9% CP, 37.6% NDF, and 39.7% starch.

[3]Alfalfa haylage was 45.8% DM and contained (DM basis) 19.7% CP, 42.7% NDF, and 2.6% starch.

[4]Straw/hay mix was 87.6% DM and contained (DM basis) 9.9% CP, 66.4% NDF, and 3.4% starch.

[5]Hydrolyzed feather meal (Papillon Agricultural Company LLC) contained 94.4% CP (DM basis). Rumen undegradable protein was 81.8% of CP, and its intestinal digestibility was 36.9% [determined in vitro according to Calsamiglia and Stern (1995) method by Rock River Laboratories Inc.].

[6]Mineral/vitamin premix (Cargill Animal Nutrition, Cargill Inc.) contained (%, as-is basis) limestone, 36.75; dry corn distillers grains with solubles, 29.00; NaCl, 24.85; MgO (54% Mg), 4.15; Bio-Phos (Rootwise), 2.45; zinc sulfate, 0.96; mineral oil, 0.5; vitamin E, 0.37; manganese sulfate, 0.37; copper sulfate, 0.26; ferrous sulfate, 0.16; Se, 0.13; vitamin A, 0.03; vitamin D_3, 0.013; calcium iodate, 0.008; cobalt carbonate, 0.005.

[7]Unless indicated otherwise, values were calculated using nutrient analysis (Cumberland Valley Analytical Services Inc.) of individual feed ingredients and their inclusion rates in the diet.

[8]Values estimated based on NRC (2001).

et al., 2011). Estimated daily urine output was used to calculate daily excretions of total N, UUN, and purine derivates (allantoin and uric acid).

Blood Sampling. Blood samples were collected from the tail vein or artery of the cows 4 times in 2 consecutive days during wk 4 of each experimental period as follows: at 1100 and 1700 h on d 1 and 1400 and 2000 h on d 2. Plasma, serum, and whole-blood samples were collected and processed as described in Räisänen et al. (2021). Composited plasma samples (1 sample per cow and per period) were analyzed for AA concentration by Ajinomoto Co Inc. using a High-Speed AA analyzer L-8900 (Hitachi High-Technologies Co.). Serum samples (collected at 1700h) were analyzed for glucagon-like peptide-1 (**GLP-1**; Bovine GLP-1 Competitive ELISA, EKB01721, Biomatik USA), neuropeptide Y (**NPY**; Bovine NPY ELISA Kit No. EKC31744, Biomatik USA), insulin (Bovine Insulin ELISA, Kit No. 10-1201-01, Mercodia AB), and histamine (pan-species ELISA, Kit No. EKU04794, Biomatik USA).

Table 2. Net energy of lactation, protein fractions, and AA supply and balance[1] in mid-lactation dairy cows fed a MP-deficient diet supplying incremental levels of digestible (d)His

	Treatment[2]			
Item	dHis1.8	dHis2.2	dHis2.6	dHis3.0
NE_L, Mcal/d				
Requirement	35.3	35.4	35.6	35.9
Supply	32.3	32.5	32.2	32.7
Balance	−3.0	−2.9	−3.4	−3.2
Protein fraction balance, g/d				
MP				
Requirement	2,294	2,372	2,395	2,421
Supply	2,046	2,057	2,037	2,075
Balance	−248	−315	−358	−346
Supplied/requirement	0.89	0.87	0.85	0.86
RDP and RUP				
RDP supply	1,946	1,956	1,938	1,970
RDP balance	−126	−125	−125	−127
RUP supply	1,392	1,536	1,385	1,414
RUP balance	−388	−552	−561	−542
AA balance, g/d				
dHis				
Supply from basal diet	37	37	37	38
Supply from RP-His[3]	—	16	26	36
Total supply	37	53	63	74
dMet				
Requirement[4]	50	52	53	53
Supply from basal diet	35	36	35	36
Supply from RP-Met[3]	21	21	21	21
Balance	6	5	3	4
dLys				
Requirement[4]	151	157	158	160
Supply from basal diet	110	111	110	112
Supply from RP-Lys[3]	36	36	36	36
Balance	−5	−10	−12	−12
Other AA supplied,[1] g/d				
dArg	87	87	86	88
dIle	95	95	94	96
dLeu	169	170	169	172
dPhe	97	97	96	98
dThr	95	95	95	96
dVal	110	110	109	111
Total digestible EAA	892	897	888	904

[1]Values were estimated using NRC (2001) based on actual averaged DMI, milk yield and composition, and BW of individual cows during last 7 d of each experimental period.

[2]Four different levels of histidine were supplied: dHis1.8 received the basal diet, and treatments dHis2.2, dHis2.6, and dHis3.0 received the basal diet top-dressed with an additional 12, 17, and 27 g/d of dHis, respectively.

[3]The amounts of dHis, dMet, and dLys from RPAA products were calculated based on the bioavailability estimations provided by the manufacturers. RP-Met = rumen-protected Met; RP-Lys = rumen-protected Lys.

[4]Requirements of dMet and dLys were assumed as 2.2 and 6.6% (respectively) of MP requirements (NRC, 2001; Schwab et al., 2005).

Rumen Sampling. Whole-rumen content samples for fermentation analyses were collected from the 8 rumen-cannulated cows in wk 4 of each experimental period on 2 consecutive days at 0, 2, 4, 8, and 12 h after feeding. The whole ruminal contents were filtered through 2 layers of cheesecloth (to obtain approximately 300–400 mL of filtrate) and immediately analyzed for pH (59000-60 pH Tester, Cole-Parmer Instrument Company). Aliquots of the cheesecloth filtrate were centrifuged at low speed (500 × *g* for 5 min at 4°C) to remove protozoa and feed particles. The low-speed supernatant was centrifuged at 20,000 × *g* for 15 min at 4°C and analyzed for NH_3 (Chaney and Marbach, 1962) and VFA concentrations (Yang and Varga, 1989).

Calculated Histidine Pools and Efficiency of Utilization

Apparent dHis recovery was calculated based on an estimated dHis intake (NRC, 2001), and measured blood and milk protein concentrations as detailed in Räisänen et al. (2021). Briefly, the blood His pool included His in blood hemoglobin, plasma-free His, and His in plasma 1-MH, 3-MH, and carnosine. The amount of His in these metabolites was based on the proportion of His molecular weight in the molecular weight of the metabolite in question. The amount of His in plasma and blood pools was calculated based on an assumed blood volume of 6.7% of BW, and plasma volume of 68% of blood volume (Reynolds, 1953). Apparent efficiency of His utilization ($\mathbf{Eff_{His}}$) was calculated as described in Lapierre et al. (2020):

$$\mathrm{Eff_{His}} = \frac{\text{sum of His in true exported proteins (g)}}{\left[\text{digestible His flow (g)} - \text{endogenous urinary loss}\right]},$$

where the sum of His in true exported proteins includes milk true protein yield and estimated scurf and metabolic fecal protein calculated as detailed in Lapierre et al. (2020).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (release 9.4, SAS Institute Inc.). Milk yield and DMI from the last 7 d of each experimental period were analyzed as a repeated measure. The best covariance structure for repeated measures was chosen based on the lowest Akaike information criterion. The statistical model included the repeated term (day), period, parity, and treatment. Parity × treatment interactions were not significant for any variable and were excluded from the final model. Square and cow within square were random effects, and all others were fixed. The rumen data were analyzed with the same model, except the repeated term was sampling time point. Milk composition, plasma AA, nutrient intake and digestibility, urinary excretions, and serum metabolites data were analyzed with the same model without the repeated term. Milk composition data were weighted averages based on the milk production at each milking. Polynomial contrasts were used to test linear, quadratic, and cubic effects of dHis dose. Differences were considered at $P \leq 0.05$, and trends were declared at $0.05 < P \leq 0.10$. Data are presented as least squares means.

RESULTS AND DISCUSSION

The diet was reconstituted at the end of the experiment (Table 1). Energy, protein, and AA supplies were estimated using NRC (2001) based on nutrient and AA composition of the feeds with actual MY, milk composition, and DMI of the cows during the experiment (Table 2). The basal diet supplied 92, 92, 90, and 91% of NE_L and 89, 87, 85, and 86% of MP requirements for dHis1.8, dHis2.2, dHis2.6, and dHis3.0, respectively. Treatments dHis1.8, dHis2.2, dHis2.6, and dHis3.0 were supplying dLys 5, 10, 12, and 12 g/d below NRC (2001) recommendations, respectively, due to the low DMI in relation to MY and milk true protein yield, and lower-than-expected RUP digestibility of the feather meal (Table 1). The relatively greater dLys supply for dHis1.8 was not reflected in plasma Lys concentrations (see discussion below) and likely did not affect the observed responses to dHis supply.

Dry Matter Intake and Production Variables

Dry matter intake was not affected (Table 3) by dHis dose in the current experiment. There was a quadratic increase in MY ($P = 0.04$) and a linear increase ($P = 0.05$) in ECM yield with increasing dHis dose.

The lack of dHis dose effect on DMI is in line with data from our previous experiment in the companion paper (Räisänen et al., 2021), as well as from Zang et al. (2019), with short-term, crossover experiments testing effects of incremental RP-His supplementation, and Lapierre et al. (2020), testing incremental levels of abomasally infused His on lactational performance and plasma AA concentrations in lactating dairy cows. The positive effect of His on DMI was reported in long-term experiments with MP-deficient diets (Lee et al., 2012a; Giallongo et al., 2015) and a long-term experiment with MP-adequate diets (Giallongo et al., 2017), indicating that the possible effect of supplemental dHis on DMI requires a longer-term dietary His deficiency, as discussed in Räisänen et al. (2021).

Table 3. Lactational performance and BW of mid-lactation dairy cows fed a MP-deficient diet supplying incremental levels of digestible (d)His

	Treatment[1]					*P*-value[3]		
Item	dHis1.8	dHis2.2	dHis2.6	dHis3.0	SEM[2]	Linear	Quadratic	Cubic
DMI, kg/d	20.2	20.5	20.3	20.7	1.73	0.50	0.89	0.55
Milk yield, kg/d	36.9	39.2	40.5	39.3	2.98	0.02	0.04	0.44
Feed efficiency,[4] kg/kg	1.87	1.96	2.04	1.97	0.085	0.02	0.08	0.32
ECM, kg/d[5]	33.9	34.3	35.0	35.2	2.38	0.05	0.99	0.65
ECM feed efficiency, kg/kg	1.65	1.68	1.70	1.71	0.055	0.19	0.86	0.87
Milk fat, %	3.59	3.30	3.33	3.41	0.206	0.05	0.006	0.55
Milk fat, kg/d	1.32	1.27	1.31	1.33	0.106	0.54	0.21	0.48
Milk true protein, %	2.79	2.78	2.81	2.84	0.071	0.08	0.34	0.68
Milk true protein, kg/d	1.02	1.08	1.10	1.11	0.066	<0.001	0.23	0.98
Milk lactose, %	4.93	4.88	4.87	4.87	0.058	0.03	0.23	0.97
Milk lactose, kg/d	1.83	1.92	1.93	1.91	0.132	0.03	0.07	0.97
MUN, mg/dL	10.8	10.6	10.5	10.3	0.382	0.16	0.86	0.96
Milk NE_L,[6] Mcal/d	25.2	25.5	26.0	26.2	1.77	0.06	0.97	0.64
SCC,[7] $\times 10^3$ cells/mL	117	158	126	159	55.7	0.56	0.91	0.81
BW, kg	580	578	573	572	15.4	0.07	0.94	0.55

[1]Treatments were basal diet (dHis1.8) or basal diet supplemented with rumen-protected (RP)His product (dHis2.2, dHis2.6, and dHis3.0, respectively). Actual dHis supply was 37, 53, 63, and 74 g/d, for dHis1.8, dHis2.2, dHis2.6, and dHis3.0, respectively. All treatments were supplemented with rumen-protected Lys (RP-Lys) and rumen-protected Met (RP-Met).

[2]Largest SEM published in table. n = 522 for DMI, n = 464 for milk yield, n = 449 for feed efficiency, n = 79 for milk composition variables (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Contrasts: linear, quadratic, and cubic effects of dHis dose.

[4]Milk yield ÷ DMI.

[5]Calculated according to Sjaunja et al. (1990).

[6]Milk NE_L (Mcal/d) = kg of milk × (0.0929 × % fat + 0.0563 × % true protein + 0.0395 × % lactose), based on NRC (2001).

[7]Statistical analysis was performed on log-transformed data.

The quadratic increase in MY by up to 3.4 kg/d from the lowest dHis dose (37 g/d) to dHis2.6 treatment (63 g/d) is in line with previous experiments in which incremental doses of His were supplied to lactating cows with low dietary RUP supply; for example, MP-deficient corn silage-based diet (Zang et al., 2019; Lapierre et al., 2020) or grass silage-based diets (i.e., supplying low RUP; Korhonen et al., 2000; Kim et al., 2001). Indeed, Zang et al. (2019) reported a 2.6 kg/d increase in MY from no RP-His supplementation to a 58 g/d dHis supply from RP-His and basal diet. Korhonen et al. (2000) observed 1.8 kg/d greater MY in cows postruminally infused with 6 g/d of His compared with no His infusion, whereas Kim et al. (2001) reported a 2.5 kg/d increase in MY when 9 g/d of His was infused intravenously. Further, there was an increase of approximately 4 kg/d in MY when cows were abomasally infused with His in 2 short-term experiments (0–22.8 g/d and 0–30.4 g/d of His in Exp. 1 and Exp. 2, respectively) with a MP-deficient corn silage-based diet supplying 33 g/d dHis (Lapierre et al., 2020).

Histidine supplementation had varying effects on ECM yields in previously reported experiments. The linear increase in ECM yield up to +1.3 kg/d (basal diet to 74 g/d of dHis from RP-His), in the current study, agrees with data from Giallongo et al. (2016) showing an increase in ECM yield from 35.5 kg/d with a MP-deficient diet supplying 57 g/d of dHis to 38.3 kg/d with the same diet supplemented with RP-His (68 g/d of dHis), RP-Met, and RP-Lys. Giallongo et al. (2017) also reported a 3 kg/d greater ECM yield in cows fed MP-adequate diets supplying 68 versus 49 g/d of dHis. Further, our companion experiment (Räisänen et al., 2021) with incremental dHis doses with a MP-adequate diet reported a 3.1 kg/d increase in ECM with RP-His. Others reported no or minor effects of His supplementation on ECM yield (Vanhatalo et al., 1999; Giallongo et al., 2015; Zang et al., 2019). The linear MY, ECM yield, and milk true protein yield responses to incremental dHis doses in the current experiment (see following section), and the lesser response with a MP-adequate diet in Räisänen et al. (2021), support previous data suggesting that His deficiency is more apparent when the cow is more reliant on microbial protein as a source of dAA (Lee et al., 2012a,b), due to the lower concentration of His (vs. Met) in microbial protein in relation to these concentrations in milk protein (Kim et al., 1999).

There was a quadratic effect ($P = 0.006$; Table 3) of dHis dose on milk fat concentration, but milk fat yield was not affected by treatment. Milk true protein concentration tended to increase linearly ($P = 0.08$), whereas milk true protein yield increased linearly ($P < 0.001$) with increasing dHis dose. Last, milk lactose

concentration decreased linearly ($P = 0.03$), whereas lactose yield increased linearly ($P = 0.03$) with increasing dHis dose.

The decrease in milk fat concentration but not yield was most likely a result of a dilution effect due to increased MY. Similar results have been observed with postruminal infusions of His (Vanhatalo et al., 1999; Korhonen et al., 2000; Kim et al., 2001) and RP-His supplementation on a MP-deficient corn silage-based diet (Giallongo et al., 2015), whereas others have not observed differences in milk fat concentration with His supplementation (Lee et al., 2012a; Giallongo et al., 2017; Zang et al., 2019). Giallongo et al. (2015) suggested that correction of His deficiency may have a decreasing effect on milk fat concentration, but this needs to be further explored as data have been inconsistent among experiments. Cant et al. (2003) suggested that the underlying mechanism for a decreased fat concentration in response to an imbalance in absorbed AA could be related to subsequent decrease in glucose, acetate, and BHB concentrations combined with a decreased mammary blood flow.

The linear response of milk true protein concentration to increasing dHis dose agrees with Huhtanen et al. (2002) and Lapierre et al. (2021), where milk true protein concentration increased linearly with increasing doses of abomasally infused His. Further, cows fed a MP-deficient corn silage-based diet supplemented with RP-His together with RP-Met and urea had a higher milk true protein concentration compared with cows with only RP-Met and urea supplementation (Giallongo et al., 2015). Several other experiments did not report a response in milk true protein concentration to His supplementation both in cows fed grass silage (Vanhatalo et al., 1999; Korhonen et al., 2000; Kim et al., 2001) and corn silage-based diets (Lee et al., 2012a; Zang et al., 2019). However, most of the abovementioned studies (Korhonen et al., 2000; Lee et al., 2012a; Zang et al., 2019) where dietary RUP supply in the basal diet was low, reported an increase in milk true protein yield as a result of a numerically higher milk true protein concentration and increased MY in cows supplemented with His. This is in congruence with the current experiment, in which we observed up to a 9% increase in milk true protein yield with increasing dHis supply from 37 g/d to 74 g/d. These results together with previous data confirm that His is a limiting AA for milk protein synthesis in lactating dairy cows fed diets that supply decreased levels of dietary RUP and are more reliant on microbial protein as a source of dAA. Further, it is worth noting that MUN concentration was not affected by dHis dose in the current experiment, which together with the linear increase in milk true protein yield indicates that part of the additional dHis supplied from all doses to the cows was utilized for milk protein production (Vanhatalo et al., 1999).

Due to the dilution effect of the linear increase in MY, milk lactose concentration decreased linearly, whereas lactose yield increased linearly. Lactose is considered to be the main drive of milk volume (Kronfeld, 1982), and therefore a greater lactose yield can be expected when an increase in milk volume is observed.

Plasma Amino Acid Concentrations and Calculated His Pools

Plasma His concentration increased linearly ($P < 0.001$) with increasing dHis dose (Table 4). Phenylalanine concentration decreased quadratically ($P = 0.05$). Of the NEAA, dHis dose decreased ($P = 0.01$) Ser and increased ($P = 0.02$) Orn concentrations linearly. There was quadratic increase ($P \leq 0.05$) in Gly, Glu, and Cit concentrations. Histidine dose had a quadratic effect ($P \leq 0.02$) on the sum of NEAA and total AA. Further, plasma concentration of 1-MH and carnosine was linearly increased ($P \leq 0.001$) by increasing dHis dose.

Calculated His pools and apparent recovery of dHis in blood and milk pools are presented in Table 5. Milk His (g/d) increased linearly ($P < 0.001$) with increasing dHis dose, whereas dHis recovery in milk (%) decreased quadratically ($P = 0.03$). Digestible His recovery in total blood His pool (%) as well as dHis recovery in total His pool (sum of total blood and milk His pools; %) decreased quadratically ($P \leq 0.02$) with dHis dose. The apparent efficiency of His utilization decreased quadratically ($P = 0.02$) with dHis dose.

The linear response of plasma His concentration to increasing dHis supply was expected; the concentration increased up to 1.9 times from the lowest to the highest dHis dose. This is a similar increase to previous His dose-response experiments with grass silage- (Korhonen et al., 2000) or MP-deficient corn silage-based diets (Zang et al., 2019). These authors observed a 2.8- and 1.8-fold increase in plasma His concentration when 0 to 6 g/d of His was abomasally infused (Korhonen et al., 2000) or when 0 to 15 g/d dHis was supplied as RP-His (Zang et al., 2019). Further, Lapierre et al. (2021) reported a 4-fold increase in plasma His concentration, from 15 μM to 22.8 μM, when 0 to 22.8 g/d of His was abomasally infused, respectively, and a 5-fold increase when 0 to 38.0 g/d of His was infused. Plasma His concentration (21.0 μM) for the control treatment supplying 37 g/d of dHis in the current experiment was similar to that observed by Lee et al. (2012a) and Giallongo et al. (2016), who reported plasma His concentrations of 21.8 and 25.8 μM in MP-deficient diets supplying 54 and 57 g/d of dHis for cows with milk yield of 38.2 and 35.3 kg/d, respectively. Further, the plasma His

concentration increased to 41.1 and 38.4 μ*M* when the diet was supplemented with RP-His to supply 12 and 9 g/d of dHis from RP-His in Lee et al. (2012a) and Giallongo et al. (2016), respectively. This is in line with our data, where the cows with the greatest dHis dose (74 g/d) had plasma His concentration of 40.0 μ*M*. The differences in the magnitude of plasma His response to supplemental His across experiments may be due to the type of basal diet, length of supplementation, dHis source (RP-His vs. L-His), and site of His delivery (top-dressed vs. abomasal infusion).

The fact that plasma His concentration did not decrease more in the current study, compared with Lee et al. (2012a) and Giallongo et al. (2016), which supplied more dHis, could be partly explained by the lower plasma concentration of 1-MH and carnosine in cows on the control treatment (dHis1.8). This indicates a metabolism of these 2 His-containing metabolites to supply His under His-deficiency (Ouellet et al., 2014; Lapierre et al., 2014), thereby masking the real supply of His (Lapierre et al., 2021). Indeed, Lapierre et al. (2014) reported a linear increase in plasma carnosine concentration with increasing postruminal dose of dHis, whereas Lapierre et al. (2021) reported a linear increase in both plasma carnosine and anserine concentrations with increasing dHis dose. Similarly, carnosine concentration increased linearly with increasing dHis dose in cows fed a MP-deficient corn silage-based diet (Zang et al., 2019).

Apparent dHis recovery in milk and blood and Eff_{His} in the current study followed a similar pattern to that in our companion study (Räisänen et al., 2021). Overall, more of the dHis was directed toward milk protein synthesis under MP deficiency in the current experiment compared with Räisänen et al. (2021), where the diets supplied adequate MP. This is line with data from Lapierre et al. (2021), who reported the calculated Eff_{His} to decrease linearly from 1.11 to 0.76 or from 1.24 to 0.59 when 0 to 22.8 g/d or 0 to 38.0 g/d of His was infused, respectively. Further, these authors stated that Eff_{His} can be used as an indicator of potential His deficiency, and target Eff_{His} should be 0.77 (Lapierre et

Table 4. Plasma AA concentration (μ*M*) in mid-lactation dairy cows fed a MP-deficient diet supplying incremental levels of digestible (d)His

	Treatment[1]					*P*-value[3]		
Item	dHis1.8	dHis2.2	dHis2.6	dHis3.0	SEM[2]	Linear	Quadratic	Cubic
Met	21.3	20.9	21.3	21.5	1.11	0.74	0.56	0.61
Lys	61.8	60.9	63.7	64.0	2.44	0.27	0.61	0.40
Thr	87.1	88.9	91.6	88.1	2.67	0.60	0.34	0.48
Arg	70.3	75.1	73.1	71.9	2.33	0.67	0.19	0.52
Ile	123	128	125	126	4.8	0.53	0.46	0.40
Leu	130	126	124	125	4.9	0.16	0.42	0.98
Val	266	273	271	265	6.0	0.84	0.20	0.87
His	21.0	27.0	35.1	40.0	1.96	<0.001	0.73	0.14
Phe	47.7	44.6	43.7	45.2	1.2	0.07	0.05	0.77
ΣEAA	829	844	848	846	18.5	0.39	0.62	0.97
Gly	302	328	322	305	14.6	0.75	0.01	0.68
Ser	112	110	107	103	4.7	0.01	0.58	0.87
Pro	89.5	94.0	90.9	89.1	3.48	0.84	0.25	0.53
Ala	265	263	268	254	18.8	0.42	0.44	0.51
Glu	63.6	72.0	66.4	65.0	2.88	0.93	0.04	0.12
Tau	30.2	31.5	29.0	30.5	1.41	0.81	0.92	0.08
Asn	33.5	34.4	34.1	32.9	1.68	0.69	0.29	0.98
Gln	196	207	202	197	5.9	0.96	0.09	0.61
Cit	62.0	69.6	69.3	67.1	2.26	0.10	0.03	0.70
Tyr	46.5	44.7	44.7	44.7	2.32	0.28	0.46	0.84
Orn	36.2	38.7	39.5	40.5	1.17	0.02	0.62	0.87
Asp	7.13	7.77	7.63	8.04	0.430	0.11	0.79	0.49
Trp	32.5	34.8	34.1	33.2	1.15	0.62	0.07	0.60
ΣNEAA	1,277	1,335	1,314	1,270	30.6	0.81	0.03	0.69
Total AA	2,108	2,179	2,163	2,116	39.3	0.85	0.09	0.80
1-Methylhistidine	5.24	5.70	5.81	6.35	0.215	0.001	0.72	0.56
3-Methylhistidine	4.91	5.06	4.95	5.01	0.220	0.71	0.70	0.48
Carnosine	8.04	9.20	9.59	9.52	0.829	<0.001	0.06	0.88

[1]Treatments were basal diet (dHis1.8) or basal diet supplemented with rumen-protected His (RP-His) product (dHis2.2, dHis2.6, and dHis3.0, respectively). Actual dHis supply was 37, 53, 63, and 74 g/d, for dHis1.8, dHis2.2, dHis2.6, and dHis3.0, respectively. All treatments were supplemented with rumen-protected Lys (RP-Lys) and rumen-protected Met (RP-Met).

[2]Largest SEM published in table. n = 79 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Contrasts: linear, quadratic, and cubic effects of dHis dose.

al., 2021). Accordingly, the optimal dHis supply in the conditions of the current experiment would be between 63 and 74 g/d.

Nutrient Digestibility and Nitrogen Utilization

Intake of dietary nutrients was similar among treatments in the current experiment (Table 6). Similarly, His dose did not affect apparent total-tract digestibility of nutrients. Nitrogen intake increased linearly ($P < 0.001$; Table 7) with increasing dHis dose, whereas excretion (g/d) of urinary N, UUN, fecal N, and total excreta N was not affected by dHis dose. Secretion of milk N (g/d) increased linearly ($P < 0.001$) with increasing RP-His supplementation. Fecal N and total excreta N excretion as percentage of N intake decreased linearly ($P \leq 0.001$) and excretion of urine N tended to decrease linearly ($P = 0.09$).

As expected, apparent total-tract digestibility of nutrients was similar among His doses as the cows were on the same basal diet, and the only difference among treatments was the amount of supplemental RP-His. These results concur with the study by Zang et al. (2019), and previous experiments from our laboratory, in which RP-His was supplemented on a MP-deficient diet (Lee et al., 2012a; Giallongo et al., 2015). In line with the greater milk protein yield discussed above, milk N secretion increased linearly with RP-His supplementation. Excretions of N, as percentage of N intake, decreased linearly with RP-His supplementation, further indicating that N from RP-His was used for milk protein synthesis by the mammary gland and taken up by other tissues, rather than metabolized and excreted as excess N.

Serum Metabolites

There was no effect of dHis on serum concentration of GLP-1 or NPY, whereas insulin concentration increased linearly ($P = 0.05$) with increasing dHis dose (Table 8). Histamine concentration increased quadratically ($P = 0.02$) with increasing dHis dose, and blood hemoglobin concentration was similar across treatments.

As discussed in Räisänen et al. (2021), GLP-1 and NPY secretion, together with other intake-regulating hormones, have been linked to circulating EAA concen-

Table 5. Estimated dHis intake, His pools, and apparent recovery of dHis in milk of mid-lactation dairy cows fed a MP-deficient diet supplying incremental levels of digestible (d)His

	Treatment[1]					*P*-value[3]		
Item	dHis1.8	dHis2.2	dHis2.6	dHis3.0	SEM[2]	Linear	Quadratic	Cubic
Estimated dHis intake,[4] g/d	34.9	46.2	53.7	62.2	5.30	<0.001	0.86	0.79
Estimated dHis supply from RP-His, g/d	—	16	26	36	—	—	—	—
Milk His,[5] g/d	28.5	29.7	30.2	30.6	1.71	<0.001	0.42	0.99
Apparent dHis recovery in milk His pool,[6] %	85.8	66.3	58.1	50.5	3.59	<0.001	0.03	0.86
Blood His pools								
Plasma His,[7] g	0.083	0.113	0.142	0.166	0.0096	<0.001	0.79	0.35
His in 3-methylhistidine,[7] g	0.020	0.021	0.020	0.021	0.0013	0.66	0.62	0.32
His in 1-methylhistidine,[7] g	0.022	0.024	0.024	0.025	0.0008	0.006	0.80	0.40
His in carnosine,[7] g	0.034	0.038	0.040	0.041	0.0036	<0.001	0.16	0.88
His in hemoglobin,[7] g	2.73	2.73	2.65	2.64	0.091	0.30	0.86	0.58
Total His blood pool,[8] g	2.88	2.91	2.88	2.91	0.105	0.83	0.98	0.68
Apparent dHis recovery in blood pool,[9] %	8.73	6.55	5.50	4.94	0.576	<0.001	0.002	0.64
Apparent dHis recovery in total His pool,[10] %	94.8	73.1	64.0	55.9	4.45	<0.001	0.02	0.88
Eff_{His}[11]	1.20	0.993	0.815	0.709	0.0548	<0.001	0.02	0.92

[1]Treatments were basal diet (dHis1.8) or basal diet supplemented with rumen-protected His (RP-His) product (dHis2.2, dHis2.6, and dHis3.0, respectively). Actual dHis supply was 37, 53, 63, and 74 g/d, for dHis1.8, dHis2.2, dHis2.6, and dHis3.0, respectively. All treatments were supplemented with rumen-protected Lys (RP-Lys) and rumen-protected Met (RP-Met).

[2]Largest SEM published in table. n = 79 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Contrasts: linear, quadratic, and cubic effects of dHis dose.

[4]Estimated using NRC (2001) based on the individual cow DMI, milk yield, and milk composition during the experiment and includes dHis from basal diet and RP-His.

[5]Calculated as Milk CP × His milk concentration, % of milk CP × MY (g/d). Milk His concentration was assumed to be 2.7% of milk CP.

[6]Calculated as Milk His (g) ÷ dHis intake (g) × 100.

[7]Calculated based on an assumed blood volume of 6.7% of BW and plasma volume of 68% of blood volume.

[8]Sum of plasma His (g), His in 1-methyl-His, 3-methyl-His, carnosine, and blood hemoglobin.

[9]Calculated as total His in blood (g) ÷ dHis intake (g) × 100.

[10]Calculated as [total His in blood (g) + milk His (g)] ÷ dHis intake (g) × 100.

[11]Apparent efficiency of His utilization: calculated as sum of His in true exported proteins (g) ÷ [dHis flow (g) – urinary excretions (g)]. For details see Material and Methods section.

Table 6. Intake and apparent total-tract digestibility of nutrients in mid-lactation dairy cows fed a MP-deficient diet supplying incremental levels of digestible (d)His

	Treatment[1]					*P*-value[3]		
Item	dHis1.8	dHis2.2	dHis2.6	dHis3.0	SEM[2]	Linear	Quadratic	Cubic
Nutrient intakes, kg/d								
DM	20.9	21.0	20.8	21.2	1.95	0.73	0.71	0.58
OM	19.6	19.7	19.5	19.8	1.82	0.74	0.71	0.59
CP	3.37	3.42	3.40	3.48	0.313	0.18	0.75	0.58
NDF	7.28	7.31	7.24	7.36	0.677	0.73	0.71	0.59
ADF	3.82	3.85	3.80	3.88	0.358	0.62	0.73	0.51
Starch	6.57	6.60	6.53	6.64	0.611	0.73	0.71	0.58
Apparent digestibility, %								
DM	65.2	64.9	65.1	65.0	0.49	0.73	0.88	0.66
OM	66.1	66.0	66.1	66.0	0.49	0.91	0.98	0.86
CP	64.5	64.3	65.8	65.4	0.75	0.25	0.97	0.25
NDF	43.3	43.2	43.3	42.9	1.06	0.72	0.80	0.83
ADF	32.8	32.8	33.1	32.5	1.58	0.90	0.77	0.81
Starch	97.9	97.7	97.8	97.9	0.01	0.88	0.23	0.44

[1]Treatments were basal diet (dHis1.8) or basal diet supplemented with rumen-protected His (RP-His) product (dHis2.2, dHis2.6 and dHis3.0, respectively). Actual dHis supply was 37, 53, 63 and 74 g/d, for dHis1.8, dHis2.2, dHis2.6 and dHis3.0, respectively. All treatments were supplemented with rumen-protected Lys (RP-Lys) and rumen-protected Met (RP-Met).

[2]Largest SEM published in table. n = 79 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Contrasts: linear, quadratic, and cubic effects of dHis dose.

trations, such as His, Arg, Asp, Ser, and Thr in nonruminant species (Haas et al., 2008; van den Broek et al., 2018). Lack of effect of dHis dose on NPY and GLP-1 concentration is in line with the data for DMI in the current experiment and data presented in Räisänen et al. (2021). More research is warranted on the role of His and other AA in feed intake regulation in ruminants.

Interestingly, insulin concentration increased linearly with increasing dHis dose. Data from our companion paper and previous experiments from our laboratory did

Table 7. Nitrogen utilization and purine derivatives (PD) excretion in mid-lactation dairy cows fed a MP-deficient diet supplying incremental levels of digestible (d)His

	Treatment[1]					*P*-value[3]		
Item	dHis1.8	dHis2.2	dHis2.6	dHis3.0	SEM[2]	Linear	Quadratic	Cubic
N intake, g/d	539	567	579	605	53.5	<0.001	0.86	0.69
N excretion or secretion, g/d								
Urinary N	169	171	157	174	9.60	0.96	0.35	0.14
Urinary urea-N	111	100	112	105	9.50	0.73	0.70	0.20
Fecal N	190	198	187	192	19.3	0.90	0.74	0.22
Total excreta N	358	368	345	366	25.2	0.95	0.62	0.06
Milk N	160	167	170	173	9.50	<0.001	0.32	0.97
N excretion and secretion	518	536	516	539	33.6	0.27	0.81	0.05
As proportion of N intake, %								
Urinary N	32.2	31.1	27.7	29.8	2.46	0.09	0.33	0.13
Urinary urea-N	21.3	18.0	19.5	18.0	1.45	0.13	0.52	0.24
Fecal N	35.4	34.4	32.3	31.7	0.67	<0.001	0.95	0.27
Total excreta N	67.5	65.6	59.9	61.5	2.62	0.001	0.38	0.05
Milk N	30.4	30.5	30.0	29.2	1.26	0.20	0.37	0.87
N excretion and secretion	93.1	95.3	88.4	91.0	4.72	0.30	0.91	0.11
Urine output, kg/d	20.9	20.3	22.4	22.5	1.49	0.17	0.65	0.28
Urinary PD excretion, mmol/d								
Uric acid	43.3	46.7	47.0	44.5	5.22	0.83	0.54	0.96
Allantoin	475	475	458	508	37.3	0.56	0.40	0.51
Total PD	519	521	505	552	40.8	0.57	0.48	0.55

[1]Treatments were basal diet (dHis1.8) or basal diet supplemented with rumen-protected (RP)His product (dHis2.2, dHis2.6, and dHis3.0, respectively). Actual dHis supply was 37, 53, 63, and 74 g/d, for dHis1.8, dHis2.2, dHis2.6, and dHis3.0, respectively. All treatments were supplemented with rumen-protected Lys (RP-Lys) and rumen-protected Met (RP-Met).

[2]Largest SEM published in table. n = 79 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Contrasts: linear, quadratic, and cubic effects of dHis dose.

Table 8. Concentration of serum hormones and blood metabolites of mid-lactation dairy cows fed a MP-deficient diet supplying incremental levels of digestible (d)His

	Treatment[1]					*P*-value[3]		
Item	dHis1.8	dHis2.2	dHis2.6	dHis3.0	SEM[2]	Linear	Quadratic	Cubic
Glucagon-like peptide-1, n*M*	26.3	23.6	25.8	26.4	1.32	0.76	0.14	0.25
Neuropeptide Y, n*M*	4.70	5.96	6.63	5.79	0.870	0.27	0.24	0.69
Insulin, p*M*	174	193	211	236	34.0	0.05	0.78	0.97
Histamine, n*M*	7.72	9.89	9.54	7.07	1.33	0.71	0.02	0.97
Hemoglobin, g/dL	8.67	8.71	8.77	8.76	0.183	0.37	0.82	0.69

[1]Treatments were basal diet (dHis1.8) or basal diet supplemented with rumen-protected (RP)His product (dHis2.2, dHis2.6, and dHis3.0, respectively). Actual dHis supply was 37, 53, 63, and 74 g/d, for dHis1.8, dHis2.2, dHis2.6, and dHis3.0, respectively. All treatments were supplemented with rumen-protected Lys (RP-Lys) and rumen-protected Met (RP-Met).

[2]Largest SEM published in table. n = 79 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Contrasts: linear, quadratic, and cubic effects of dHis dose.

not report effects of MP-level, RP-His, or other RPAA supplementation on insulin concentration (Giallongo et al., 2015, 2016, 2017). On the other hand, insulin together with EAA has been linked to upregulation of milk protein synthesis in the mammary gland through its action on the mTOR signaling pathway (Cant et al., 2018). This is in line with the linear increase in milk true protein yield observed in the current experiment. Further, in situations with low overall EAA supply, insulin upregulates mammary blood flow, thereby increasing the availability of EAA to be taken up by mammary gland (Bequette et al., 2001). This may have partly enhanced milk protein synthesis in the current experiment and contributed to the linear response in milk true protein yield to incremental dHis supply.

Similar to the data presented in our companion paper (Räisänen et al., 2021), histamine concentration increased with increasing dHis dose. Histidine can be converted to histamine by cleavage of its carboxyl group by histidine decarboxylase, and thereby the quadratic increase in histamine concentration with increasing dHis dose can be expected. Different functions of histamine are well described in monogastric animals, and include a role in immune response, gastric acid secretion, and action as a neurotransmitter in the brain (Brosnan and Brosnan, 2020). To the best of our knowledge, the concentration of histamine in response to His supplementation in ruminants has not been reported previously. Therefore, the effects of increased serum histamine concentration in ruminants are not known and need further investigation. However, microbial histidine decarboxylase is active in the rumen, and dietary His can be converted to histamine by the rumen microbes (Sanford, 1963). Histamine in turn has been shown to decrease rumen pH, therefore having implications in rumen acidosis and laminitis in dairy cows (Nocek, 1997), as discussed below.

Ruminal pH, and VFA and Ammonia Concentrations

Ruminal pH tended to linearly decrease ($P = 0.06$), whereas ammonia concentration and individual and total VFA concentrations were not affected by dHis supplementation (Table 9). The molar proportion of acetate and isovalerate increased ($P \leq 0.02$) linearly with dHis dose, whereas the molar proportion of propionate and butyrate decreased ($P \leq 0.002$) linearly. There was a linear tendency for decreased molar proportion of isobutyrate ($P = 0.06$).

As the experimental diets in the current study were supplemented with a RP product, ruminal effects of treatment were expected to be subtle. Only a few studies reported effects of His on rumen fermentation. The in vivo studies have been done with cows in which His was infused postruminally (Vanhatalo et al., 1999; Korhonen et al., 2000; Huhtanen et al., 2002) and reported biologically nonsignificant effects of His on rumen fermentation. In the current experiment, His was supplemented in the form of RP-His, which means that part of the His in the product was likely released in the rumen, before passing through to the lower digestive tract. The manufacturer of the product used in the current experiment reported a rumen escape fraction of 90%, which means that around 3, 5, and 7 g of His for dHis2.2, dHis2.6, and dHis3.0, respectively, may have been released in the rumen from the RP-His product. Further, because the product is coated with hydrogenated vegetable oil rich in stearic acid (C18:0), some C18:0 from the coating may have been released in the rumen (around 3.3, 5.6, and 7.8 g for dHis2.2, dHis2.6, and dHis3.0, respectively). This additional His and C18:0 had minor or no effects on rumen fermentation.

Most notably, pH tended to be decreased with increasing dHis dose, being up to 1.1 units lower for the highest dHis dose compared with control. This may

Table 9. Rumen fermentation parameters of mid-lactation dairy cows fed a MP-deficient diet supplying incremental levels of digestible (d)His

	Treatment[1]					*P*-value[3]		
Item	dHis1.8	dHis2.2	dHis2.6	dHis3.0	SEM[2]	Linear	Quadratic	Cubic
pH	6.32	6.28	6.25	6.21	0.093	0.06	0.88	0.94
NH_3, m*M*	5.49	5.43	5.79	5.37	0.376	0.98	0.60	0.38
VFA, m*M*								
Acetate	79.0	80.5	79.2	79.7	1.38	0.85	0.72	0.50
Propionate	28.3	27.4	26.9	27.5	2.42	0.39	0.37	0.77
Isobutyrate	0.82	0.80	0.81	0.79	0.018	0.25	0.94	0.49
Butyrate	14.8	14.7	15.0	14.4	0.523	0.69	0.65	0.66
Isovalerate	2.08	2.08	2.05	1.89	0.191	0.16	0.36	0.80
Valerate	1.75	2.06	1.92	1.81	0.173	0.88	0.14	0.52
Total VFA, m*M*	127	128	126	126	3.70	0.72	0.85	0.60
VFA, mol %								
Acetate	61.4	64.2	61.6	64.4	0.71	0.007	0.90	<0.001
Propionate	22.7	20.7	22.9	20.5	0.37	0.006	0.72	<0.001
Isobutyrate	0.61	0.63	0.64	0.68	0.050	0.06	0.53	0.89
Butyrate	12.2	11.5	11.8	11.1	0.49	0.002	0.97	0.03
Isovalerate	1.56	1.54	1.55	1.76	0.053	0.02	0.03	0.53
Valerate	1.50	1.41	1.51	1.52	0.033	0.47	0.12	0.11
Acetate:Propionate	2.87	2.97	2.98	2.98	0.21	0.24	0.45	0.83

[1]Treatments were basal diet (dHis1.8) or basal diet supplemented with rumen-protected His (RP-His) product (dHis2.2, dHis2.6, and dHis3.0, respectively). Actual dHis supply was 37, 53, 63, and 74 g/d, for dHis1.8, dHis2.2, dHis2.6, and dHis3.0, respectively. All treatments were supplemented with rumen-protected Lys (RP-Lys) and rumen-protected Met (RP-Met).

[2]Largest SEM published in table. n = 160 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Contrasts: linear, quadratic, and cubic effects of dHis dose.

have been a result of a linear decrease in the proportion of butyrate in cows supplemented with RP-His, as butyrate synthesis leads to increased pH due to enhanced proton removal through the rumen wall (Penner et al., 2009), as discussed in Melgar et al. (2020). Histamine has been shown to decrease ruminal pH and plays a role in rumen acidosis and laminitis in cattle (Nocek, 1997). The decreased pH with increasing dHis dose in the current experiment may have partly been a result of increased histamine concentration in the rumen, as histamine concentration in serum increased quadratically with increasing dHis dose (see above). However, it is noted that the amount of His released in the rumen was relatively small as stated above. Interestingly, the increase in the molar proportion of acetate and decrease in the molar proportion of propionate would warrant the opposite effect on pH (Russell, 1998). However, relative changes in concentrations of these VFA were inconsistent and small and most likely did not contribute to the change in pH.

There is evidence that NAN and AA enhance microbial protein synthesis and growth in the rumen and affect rumen fermentation pattern (Argyle and Baldwin, 1989; Clark et al., 1992, Guliye et al., 2005), but specific effects of His on rumen fermentation have not been investigated. Stearic acid from the fat coating of RP-His most likely had little effect on the molar proportions of VFA, as it is inert in the rumen (Loften et al., 2014), and previous research reported minor effects on rumen fermentation (Chalupa et al., 1986).

CONCLUSIONS

In accordance with our hypothesis, ECM yield, milk true protein yield, and plasma His concentration increased linearly with increasing dHis dose in lactating cows fed a basal diet that was 10 to 15% deficient in MP, according to NRC (2001). Dry matter intake, however, was not affected by incremental levels of dHis in this short-term experiment, whereas lactational performance was optimized at dHis supply of 74 g/d (or 3.0% of MP). Data from the current experiment indicates that MP-deficiency amplifies the production responses to dHis supply.

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