Milk protein yield and mammary metabolism are affected by phenylalanine deficiency but not by threonine or tryptophan deficiency

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ABSTRACT

Efficient milk protein synthesis requires that the essential AA be presented to the mammary gland in the right amount and proportion to maximize protein synthesis and minimize losses. This study investigated the effects of individual AA deficiencies on cow productivity, mammary metabolism, and glucose whole-body rate of appearance. Five Holstein cows were used in a 5 × 5 Latin square design trial with 10-d periods. Treatments were abomasal infusions of (1) water (CTL); (2) complete AA mixture (TAA); (3) TAA without Phe (No-Phe); (4) TAA without Thr (No-Thr); and (5) TAA without Trp (No-Trp). Each treatment was compared with TAA. Treatment did not affect milk, fat, or lactose yields. Arterial concentrations of Phe, Thr, and Trp decreased with their respective deletions by 60, 76, and 69%. In response to the decreased arterial supply of the deleted AA, mammary plasma flow significantly increased by 55% with No-Thr but did not increase with No-Phe or No-Trp. Mammary uptake of Phe was reduced by No-Phe, accompanied by a reduced milk protein yield; uptakes of Thr and Trp were not affected by their respective deletions, and milk protein yield did not decrease with these treatments. Deletion of Phe tended to reduce its mammary uptake relative to milk output (U:O), accompanied by an increased U:O of Tyr, but deletion of Thr and Trp did not affect the U:O of the corresponding AA. Plasma urea-N concentration was lower with CTL and tended to be higher with No-Phe. Arterial concentrations and mammary uptake of acetate, β -hydroxybutyrate, glucose, and lactate were unaffected by treatment. Treatment had no effect on glucose rate of appearance at the whole-body level. Lactose output as a percentage of glucose whole-body rate of appearance was not affected by treatment. Overall,

the study indicated that a deficiency of Phe negatively affected productivity and mammary metabolism but that a deficiency of Thr or Trp did not.

Key words: phenylalanine, threonine, tryptophan, mammary, glucose

INTRODUCTION

The most effective strategy to increase the efficiency of nitrogen utilization in dairy cows is to decrease the amount of N fed (Doepel et al., 2004; Kebreab et al., 2010), but this strategy can be used on farms only to the extent that it can be done without detrimental effects on milk production. Reducing CP intake, and therefore the margin of safety on protein supply, might create deficiencies in the supply of some AA, which could have a negative effect on milk and milk protein yields; hence, the increased need to properly balance AA supply with demand. Milk and milk component yields respond strongly to deletion of the EAA from an infused total AA mixture, but do not respond to deletion of the NEAA (Metcalf et al., 1996; Doepel and Lapierre, 2010). Lysine and Met are considered first- and second-limiting AA in corn-based diets (NRC, 2001). On grass silage-based diets, His was reported to be first-limiting (Varvikko et al., 1999), although this limitation might be more related to the high proportion of microbial protein in the MP supply than in grass silage per se (Lee et al., 2012). The majority of AA research in dairy cows has focused on these 3 AA plus the branched-chain AA (BCAA; e.g., Weekes et al., 2006); however, the other EAA also need to be supplied by the digested protein to sustain milk production. Although Phe, Thr, and Trp have received minimal attention, the importance of these AA should not be overlooked. Indeed, in a series of studies conducted in the 1970s, addition of Thr to an AA mixture increased milk yield, and addition of His, Phe, and Thr to an AA infusion increased milk protein concentration (Schwab et al., 1976). Recently, Doelman et al. (2015) reported that deletion of Phe from an AA mixture infusion decreased milk protein yield by 24%, whereas deletion of Trp had no effect compared with the complete EAA infusion.

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When additional AA are supplied postruminally, an increase in milk lactose yield occurs that is almost identical to that of milk protein yield (Lapierre et al., 2010). This suggests a close interaction between AA supply and glucose availability, utilization, or both. Indeed, when extra AA have been supplied postruminally, glucose availability at the whole-body level (measured as whole-body rate of appearance: **WBRA**) increases (Lemosquet et al., 2009; Lapierre et al., 2010). Amino acids may contribute to increased glucose WBRA through 2 mechanisms: altered absorption and gluconeogenesis. The contribution from AA to glucose availability via hepatic gluconeogenesis is estimated to vary between 12 and 20% (Reynolds et al., 2003; Lapierre et al., 2010). In cows in established lactation, the increase in glucose WBRA in response to a free AA or casein infusion could be completely accounted for by the increase in hepatic net flux of glucose (Galindo et al., 2011). On the other hand, deletion of His, Met, or Lys from an AA mixture infused abomasally increased circulating glucose concentrations in dairy cows (Weekes et al., 2006). Because deletion of the 3 AA decreased milk protein yield compared with the total AA mixture, the increased glucose concentration might reflect that AA supply not used for milk protein synthesis was used to support gluconeogenesis without a concomitant increase in utilization because lactose secretion was unaffected by treatments.

Our hypothesis was that deletion of Phe, Thr, or Trp from an AA mixture infused into the abomasum would decrease milk and milk protein yields, and concomitantly increase glucose WBRA. Therefore, the objectives of this study were to determine how milk and milk component yields change when the supply of Phe, Thr, or Trp is decreased, and to determine the relationship between these AA deficiencies and glucose availability and mammary uptake of AA and energy-yielding nutrients.

MATERIALS AND METHODS

Feeding and Treatments

The experiment was carried out at the Dairy Research and Technology Centre at the University of Alberta (Edmonton, AB, Canada). Animals were cared for in accordance with the Canadian Council on Animal Care (2009), and the Animal Care and Use Committee of the University of Alberta approved all experimental procedures. Five second-lactation Holstein cows averaging 545.8 ± 15.5 kg were used. They were housed in a tiestall barn, had free access to water, and were milked at 0400 and 1500 h. At 38 ± 1.6 DIM, 2 abomasal

catheters were implanted in each cow, as described by Doepel et al. (2006), and treatments were initiated 25 d later.

The experimental design was a 5×5 Latin square with 10-d periods. To avoid treatment carryover effects between periods and to allow for sampling on consistent days of the week, each period was followed by 4 d in which the cows did not receive treatment. The experimental treatments were abomasal infusions of (1) water (control, **CTL**); (2) all AA supplied at a rate such that the diet plus the infusion supplied 100% of the cow's MP requirement (as determined by NRC, 2001) (**TAA**); (3) all AA without Phe (**No-Phe**); (4) all AA without Thr (**No-Thr**); or (5) all AA without Trp (**No-Trp**). The AA composition of the infusion was the same as that in milk casein (Galindo et al., 2011) except that part of the Glu was replaced with Gln due to solubility limitations (Table 1). The infusates were prepared every 2 to 3 d; AA were dissolved in 15 L of water and infused continuously via peristaltic pump at a rate of 625 mL/h.

A TMR was formulated to supply 100% of the net energy requirement and 66% of the MP requirement for a second-lactation cow weighing 550 kg and producing 40 kg of milk/d with 3.6% fat and 3.2% protein (NRC, 2001; Table 2). At the start of the study, both DMI and milk yield were lower than predicted; the NRC-predicted DMI was 22.6 kg/d but the cows were

Table 1. Daily amount (g) of AA infused abomasally into the cows

AA	TAA ¹
Ala	27.7
Arg	33.3
Asn	34.3
Asp	27.8
Cys	3.6
Gln	135.0
Glu	52.9
Gly	16.7
His	24.8
Ile	44.8
Leu	83.8
Lys	70.5
Met	26.0
Phe	46.3
Pro	95.5
Ser	51.4
Thr	39.0
Trp	10.8
Tyr	49.6
Val	51.0
Total	924.8

¹The TAA treatment included all AA supplied at a rate such that the diet plus the infusion supplied 100% of the cow's MP requirement (as determined by NRC, 2001); for No-Phe, No-Thr, and No-Trp treatments, the respective AA was deleted from the infusate.

eating approximately 18 kg/d and milk yield (36 kg) was proportionally lower. As a result, the diet provided 68% of the MP requirement for the cow's milk production at the start of the study. For 20 d preceding the study, cows were fed the TMR *ad libitum* plus 2.5 kg of soybean meal per day to reduce the limitation of MP and the impairment of milk protein synthesis before the start of the experiment. During the study, cows were limited to their average DMI measured during the last 4 d before initiation of the treatments to avoid confounding effects of DMI and treatment. During the 4-d inter-experimental periods, the TMR was again top-dressed with 2.2 kg of soybean meal/cow per day so that the diet more closely met the cows' MP requirements. The cows were fed once daily except for the last 4 d of each experimental period, when they were fed equal meals every 4 h to reduce daily fluctuations in intestinal AA flow and plasma AA concentrations.

Sampling and Laboratory Analysis

Feed amounts offered and refused were recorded daily. Moisture content of the ingredients was determined weekly and the rations adjusted to maintain constant delivery of DM. Ingredient samples were collected weekly, pooled by period, and stored at -20°C until analyzed. In addition, on the last 3 d of each experimental period, orts samples were collected. Ingredient and orts samples were dried in a forced-air oven at 55°C for 72 h. The ingredient samples were then ground through a Wiley mill no. 02 (1-mm screen; Arthur H. Thomas, Philadelphia, PA) and sent to a commercial laboratory (Dairy One, Ithaca, NY) for nutrient analysis by wet chemistry procedures. Milk samples, collected on the last 3 d of each period from the morning and afternoon milkings, were analyzed for protein, fat, lactose, and MUN by infrared spectroscopy (Milk-O-Scan 605; Foss Electric, Hillerød, Denmark) at the Canwest Central Milk Testing Laboratory (Edmonton, AB, Canada).

From 1030 to 1430 h on d 9 of each period, $[6,6\text{-}^2\text{H}_2]$ glucose [99 mol percent excess (**MPE**); Cambridge Isotope Laboratories, Andover, MA] was infused into 1 jugular vein at a rate of 20 mmol/h. Blood samples were collected into heparinized tubes from the contralateral jugular vein, 1 preinfusion and then every 30 min from 1230 to 1430 h ($n = 5$). Samples were centrifuged at $1,800 \times g$ at 4°C for 25 min. To determine glucose concentrations, 1 g of plasma was mixed with 0.2 g of $[\text{U-}^{13}\text{C}]$ glucose (16 mM; 98% MPE, Cambridge Isotope Laboratories). Processed and raw plasma were stored at -80°C until analysis.

On d 10 of each experimental period starting at 0430 h, blood samples were collected every 2 h from

Table 2. Ingredient and nutrient composition (% of DM unless otherwise noted) of the TMR

Item	TMR
Ingredient	
Alfalfa hay	10.0
Barley silage	42.0
Corn grain, rolled	24.7
Barley grain, rolled	10.1
Barley grain, ground	5.1
Wheat distillers grains	2.1
Ca soap of fatty acids ¹	2.0
Tallow	1.3
Molasses	1.1
Sodium bicarbonate	0.71
Dicalcium phosphate	0.36
Vitamin/mineral premix ²	0.18
Salt	0.18
Magnesium oxide	0.18
Nutrients	
NE _L , ³ Mcal/kg	1.59
CP, %	12.0
ADF, %	19.3
NDF, %	31.6
Ca, %	0.71
P, %	0.39
Mg, %	0.26
K, %	1.62

¹Megalac (Church & Dwight Co. Inc., Princeton, NJ).

²Supplied the following per kilogram of diet: 1.19 mg of Co, 36.9 mg of Cu, 1.44 mg of I, 64.8 mg of Mn, 0.30 mg of Se, 109.7 mg of Zn, 6,770 IU of vitamin A, 1,590 IU of vitamin D, 40 IU of vitamin E.

³Calculated from NRC (2001)-predicted DMI of 22.6 kg/d.

a coccygeal vessel (arterial blood) and subcutaneous abdominal vein (venous blood) by venipuncture into sodium heparinized tubes ($n = 6$). Samples were immediately placed on ice and centrifuged for 25 min at $1,800 \times g$ at 4°C within 1 h of collection. Plasma for acetate, lactate, urea, and BHB analyses was stored at -20°C . For glucose analysis, 1 g of plasma was mixed with 0.2 g of $[\text{U-}^{13}\text{C}]$ glucose (16 mM; 98% MPE, Cambridge Isotope Laboratories). Similarly, for AA analysis, 1 g of plasma was added to 0.2 g of an internal AA standard as described by Doepel and Lapierre (2010). The samples were stored at -80°C until analyzed by the isotope dilution method of Calder et al. (1999), as described in Maxin et al. (2013). Individual samples were used for all analyses.

Plasma acetate concentrations were determined as described by Lemosquet et al. (2009). Plasma lactate was measured using an enzymatic assay (LACT2, Cobas c501, Hoffman-La Roche, Mississauga, ON, Canada), and BHB was analyzed using an Olympus AU680 Chemistry Analyzer (Beckman Coulter, Mississauga, ON, Canada). Blood urea nitrogen was determined calorimetrically (Urea Nitrogen 0580, Stanbio, Boerne, Texas). Glucose enrichment and concentration were determined through monitoring of ions 242 and 244 (for

the infused [6,6-²H₂]glucose) and ions 242 and 247 (for the [U-¹³C]glucose added to the samples as the internal standard) as described by Galindo et al. (2011).

Calculations

Digestible duodenal EAA flow, excluding that of Trp, was calculated using NRC (2001). Tryptophan flow was calculated as digestible RUP (g/d from NRC, 2001) × Trp (% of CP from NRC, 2001) + microbial MP (g/d from NRC, 2001) × Trp concentration [M. Sok (Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada) and H. Lapierre, personal communication] + endogenous MP (g/d from NRC, 2001) × Trp concentration (Lapierre et al., 2014). Energy-corrected milk was calculated as follows: ECM = [(12.86 × kg of fat) + (7.04 × kg of protein) + (0.3246 × kg of milk)] (Maltz et al., 2013). Milk AA output in protein originating, on a net basis, from mammary synthesis from free plasma AA was calculated using true milk protein yield measured on the last day of each period with a 3.37% correction for blood-borne proteins and AA composition as specified by Lapierre et al. (2012). Mammary plasma flow (**MPF**) was estimated according to the Fick principle using Phe and Tyr as internal markers (Cant et al., 1993) and an allowance for blood-borne proteins: MPF = [(milk Phe + Tyr) × 0.9663]/(arterio-venous difference Phe + Tyr) (Lapierre et al., 2012). Although Phe deficiency was one of the treatments, use of the Fick principle is still considered valid, as discussed by Lapierre et al. (2012). Net fluxes of nutrients across the mammary gland were calculated for each cow period as arterio-venous concentration difference (**AVdiff**) × MPF. Because the plasma isotopic enrichment (**IE**) of glucose had not reached a plateau at the initiation of the blood sampling, the WBRa of glucose was calculated using a non-steady-state model, as proposed by Brockman (1984). Briefly, the WBRa of glucose for each cow × period was estimated as the average of the following calculation, estimated for each sampling interval:

$$\text{WBRa} = \frac{F - p \times V \times [A] \times \Delta\text{IE}/\Delta t}{\text{IE}} - F,$$

where WBRa is expressed in mmol/h; F is the rate of infusion (mmol/h) of [6,6-²H₂]glucose; [A] (mM) and IE (MPE) are the means of plasma concentration and IE, respectively, over the time interval; ΔIE (MPE) is the variation of plasma [6,6-²H₂]glucose IE; and Δt is the time interval for which Ra was calculated; V represents the distribution volume of glucose and was estimated

as 110 mL/kg BW (Lemosquet et al., 2004), and a pool fraction $p = 0.65$ was used, as proposed by Brockman (1984).

Statistical Analysis

Dry matter intake, milk yield, and milk composition data were averaged over the last 3 d of each period. Metabolite concentrations were averaged over the 6 sampling times on d 10 of each period. The abomasal cannula became plugged in 1 cow during the third period so she was not used for periods 3, 4, and 5. In addition, 1 cow had very low DMI for period 5, so her data were not included in the statistical analyses. Finally, $n = 4$ for all treatments except for TAA ($n = 5$). Data were analyzed for normality using Proc Univariate in SAS (SAS Institute Inc., Cary, NC). The Shapiro-Wilk test was used to test Student residuals; data with residuals with $P > 0.05$ were considered normally distributed. Data were analyzed using the MIXED procedure of SAS with treatment as the fixed effect and period and cow as random effects. To test the effect of single EAA deficiency, which was the objective of the current study, each treatment was compared with TAA; treatment means were separated by the Dunnett test. Significance was declared at $P \leq 0.05$ with a tendency at $0.05 < P \leq 0.10$. All results are reported as least squares means with standard errors of the mean.

RESULTS

Nutrient Supply and Milk Yield

Dry matter intake and nutrient supply derived from the basal diet are shown in Table 3. Digestible AA flow from the basal diet in the TAA treatment was 1,355 g/d, whereas the infusion of free AA was 804 g/d when calculated on a protein basis (removing the molecule of water lost with the peptide bond). The infusion therefore accounted for 37% of the MP supply, and 38, 33, and 34% of the digestible flow of Phe, Thr, and Trp, respectively, for the TAA treatment. For TAA, digestible Phe, Thr, and Trp were 5.1, 4.8, and 1.3% of MP, whereas for the No-Phe, No-Thr, and No-Trp treatments, digestible Phe, Thr, and Trp were 3.2, 3.2, and 0.9% of MP.

Because cows were restricted to their pre-experimental DMI amounts, DMI was not different between the AA-deficient treatments and TAA ($P > 0.10$). In line with DMI, NE_L and MP supply and digestible AA flows from the diet were unaffected by treatment.

Milk and ECM yields were not different among the treatments (Table 4). Milk protein yield was lower (P

Table 3. Dry matter and NE_L intake, MP supply, and digestible AA flow

Item	Treatment ¹					SEM	P-value ²			
	CTL	No-Phe	No-Thr	No-Trp	TAA		TAA-Ctl	TAA-NoPhe	TAA-NoThr	TAA-NoTrp
DMI, kg/d	17.8	17.0	17.6	17.2	18.0	0.60	0.98	0.26	0.86	0.47
Supplied from diet										
NE _L , Mcal/d	29.8	28.8	29.5	29.1	30.1	0.83	0.98	0.27	0.85	0.46
MP, g/d	1,340	1,281	1,325	1,300	1,355	46.0	0.98	0.27	0.86	0.48
Supplied from infusion										
NE _L , Mcal/d	0	2.09	2.11	2.18	2.20					
MP, g/d	0	764	770	795	804					
Digestible AA flow from the diet, ³ g/d										
Arg	59	56	59	57	59	1.9	0.97	0.28	0.96	0.54
His	27	26	27	26	27	0.9	0.99	0.33	0.89	0.44
Ile	70	67	70	68	71	2.2	0.95	0.23	0.90	0.42
Leu	109	104	108	106	110	3.6	0.98	0.22	0.88	0.44
Lys	92	88	91	89	93	2.9	0.99	0.26	0.85	0.41
Met	27	26	27	26	27	0.8	0.88	0.22	0.81	0.33
Phe	68	65	67	66	69	2.2	0.98	0.21	0.77	0.42
Thr	69	66	68	67	70	2.2	0.99	0.29	0.90	0.52
Trp	18	18	18	18	18	0.6	1.00	0.38	0.98	0.70
Val	81	78	81	79	82	2.6	0.98	0.27	0.88	0.46

¹CTL = water infusion; No-Phe = all AA infused excluding Phe; No-Thr = all AA infused excluding Thr; No-Trp = all AA infused excluding Trp; TAA = all AA infused.

²P-values: each treatment was compared with TAA using the Dunnett test; TAA-Ctl = TAA vs. CTL; TAA-NoPhe = TAA vs. No-Phe; TAA-NoThr = TAA vs. No-Thr; TAA-NoTrp = TAA vs. No-Trp.

³Derived from NRC (2001) except Trp; see text for details.

≤ 0.04) for CTL and No-Phe relative to TAA but was unaffected by No-Thr and No-Trp. Treatment did not affect milk protein percentage. Milk fat (1,128 g/d) and lactose (1,428 g/d) yields, and milk fat and lactose percentages were not affected by treatment. Milk urea-N was higher for TAA than for CTL ($P < 0.01$) but was not different among the other treatments and TAA.

Urea and AA

Plasma urea-N concentration was lower with CTL ($P < 0.01$) compared with TAA and tended to be higher ($P = 0.09$) with No-Phe relative to TAA (Table 5). As expected, arterial concentrations of Phe, Thr, and Trp decreased ($P < 0.01$) with their respective deletions

Table 4. Effect of AA infusions on milk production and composition

Item	Treatment ¹					SEM	P-value ²			
	CTL	No-Phe	No-Thr	No-Trp	TAA		TAA-Ctl	TAA-NoPhe	TAA-NoThr	TAA-NoTrp
Yield										
Milk, kg/d	29.9	30.1	30.4	32.8	33.4	2.13	0.28	0.33	0.38	0.99
ECM, kg/d	28.5	29.1	30.7	32.9	31.7	1.62	0.29	0.45	0.94	0.89
Fat, g/d	1,038	1,077	1,156	1,246	1,123	69.8	0.73	0.95	0.98	0.46
Protein, g/d	781	771	843	883	914	43.7	0.04	0.03	0.34	0.87
Lactose, g/d	1,360	1,381	1,391	1,506	1,502	93.5	0.40	0.52	0.58	0.99
Milk composition										
Fat, %	3.58	3.59	3.92	3.80	3.41	0.23	0.85	0.84	0.13	0.29
Protein, %	2.63	2.57	2.83	2.70	2.75	0.13	0.76	0.51	0.93	0.98
Lactose, %	4.55	4.59	4.59	4.60	4.50	0.05	0.63	0.15	0.16	0.13
MUN, mg/dL	8.8	13.5	14.6	14.2	14.1	0.94	<0.01	0.97	0.98	0.99

¹CTL = water infusion; No-Phe = all AA infused excluding Phe; No-Thr = all AA infused excluding Thr; No-Trp = all AA infused excluding Trp; TAA = all AA infused.

²P-values: each treatment was compared with TAA using the Dunnett test; TAA-Ctl = TAA vs. CTL; TAA-NoPhe = TAA vs. No-Phe; TAA-NoThr = TAA vs. No-Thr; TAA-NoTrp = TAA vs. No-Trp.

(Table 5). With the exception of Lys, Met, and Trp, EAA concentrations decreased, or tended to decrease, with CTL relative to TAA. The deletions of single AA did not affect the concentrations of other EAA. With regard to NEAA, concentrations of Gln, Pro, and Tyr decreased ($P < 0.01$) with CTL, Cys decreased ($P \leq 0.01$) with No-Thr and No-Trp, and Ser increased ($P = 0.03$) with No-Trp compared with TAA.

Mammary plasma flow was increased ($P < 0.01$) by the No-Thr treatment compared with TAA, whereas MPF with the other treatments did not differ from TAA (Table 6). Compared with TAA, the CTL treatment reduced ($P \leq 0.05$) mammary uptake of Ile, Leu, Val, and Tyr, and tended to reduce ($P = 0.06$) uptakes of Phe and Pro. Uptake of Phe was reduced ($P < 0.01$) by the No-Phe treatment relative to TAA but uptakes of Thr and Trp were not affected by their respective deletions (Table 6). The single AA deletions did not affect mammary uptake of the remaining EAA. The No-Phe treatment tended to increase the uptake of Cys ($P = 0.08$). The No-Thr treatment decreased the uptake of Ser ($P = 0.02$) and tended to reduce the uptake of Gly, Pro, and Tyr ($0.05 < P \leq 0.10$), and increase the uptake of Glu ($P = 0.07$). The No-Trp treatment did not affect uptake of any AA. Treatment had no effect on the uptake of Group-1 AA-N, Group-2 AA-N, the total

EAA (**TEAA-N**), the total NEAA (**TNEAA-N**), or the total of all AA (**TAA-N**).

Compared with TAA, the mammary uptake to milk protein output (**U:O**) ratio of CTL decreased ($P < 0.01$) for the BCAA. The U:O ratio was higher ($P \leq 0.03$) for Ile, Leu, BCAA, Group-2 AA-N, and TEAA-N for No-Phe and No-Thr compared with TAA (Table 7). Deletion of Phe tended ($P = 0.09$) to reduce its U:O ratio but this was offset by an increase ($P = 0.02$) in U:O ratio of Tyr. Deletions of Thr and Trp did not affect the U:O ratio of the corresponding AA. The No-Trp treatment had minimal effects on U:O ratios of the EAA, with only Lys having an increase ($P = 0.02$). The changes in the U:O of the NEAA were not consistent across treatments, with no differences in the ratios between No-Trp and TAA, and increases for Glu ($P \leq 0.02$) for CTL, No-Phe and No-Thr versus TAA. The U:O ratio of Ser decreased ($P = 0.02$) and Gly and Tyr tended to decrease ($P \leq 0.10$) with the No-Thr treatment.

Energy-Yielding Nutrients

Plasma concentrations of acetate, BHB, glucose, and lactate were measured as indicators of energy status of

Table 5. Effect of AA infusions on plasma arterial concentrations of AA (μM) and urea-N (mM)

Item	Treatment ¹					SEM	P-value ²			
	CTL	No-Phe	No-Thr	No-Trp	TAA		TAA- Ctl	TAA- NoPhe	TAA- NoThr	TAA- NoTrp
EAA										
His	22.8	61.8	56.9	55.6	62.6	4.62	<0.01	0.99	0.72	0.59
Ile	92.7	137.0	117.6	131.3	119.4	8.11	0.09	0.31	0.99	0.62
Leu	112.6	219.1	196.0	218.2	186.3	12.92	<0.01	0.25	0.94	0.27
Lys	57.7	71.2	64.9	69.9	63.8	6.44	0.87	0.78	0.99	0.87
Met	26.5	32.2	32.0	31.7	32.5	2.20	0.17	0.99	0.99	0.99
Phe	46.8	21.5	58.5	58.3	54.0	2.67	0.06	<0.01	0.28	0.33
Thr	72.3	117.7	25.3	109.2	106.7	8.98	0.04	0.73	<0.01	0.99
Trp	38.3	46.9	44.8	13.3	42.8	3.03	0.29	0.38	0.85	<0.01
Val	171.3	318.0	284.3	311.7	266.0	15.82	<0.01	0.12	0.81	0.18
NEAA										
Ala	193.4	176.9	169.3	175.7	181.3	11.88	0.79	0.99	0.80	0.98
Asn	31.0	38.8	37.9	38.2	37.6	2.82	0.27	0.99	0.99	0.99
Asp	25.2	18.4	20.0	17.6	18.6	4.29	0.52	1.00	0.99	0.99
Cys	104.5	105.5	91.3	83.6	111.2	6.25	0.50	0.62	0.01	<0.01
Gln	182.8	258.6	252.0	215.5	243.7	16.53	<0.01	0.68	0.93	0.20
Glu	69.1	64.1	59.3	56.2	61.3	4.33	0.15	0.84	0.94	0.44
Gly	211.2	211.9	216.0	242.9	191.8	23.06	0.76	0.74	0.61	0.11
Pro	76.6	198.8	188.8	183.1	179.9	18.11	<0.01	0.46	0.90	0.99
Ser	74.4	106.1	114.0	125.1	83.8	13.00	0.86	0.28	0.11	0.03
Tyr	45.8	73.0	76.7	75.8	71.3	4.16	<0.01	0.99	0.62	0.74
Urea-N	6.8	13.2	11.5	11.6	11.1	0.73	<0.01	0.09	0.98	0.94

¹CTL = water infusion; No-Phe = all AA infused excluding Phe; No-Thr = all AA infused excluding Thr; No-Trp = all AA infused excluding Trp; TAA = all AA infused.

²P-values: each treatment was compared with TAA using the Dunnett test; TAA-Ctl = TAA vs. CTL; TAA-NoPhe = TAA vs. No-Phe; TAA-NoThr = TAA vs. No-Thr; TAA-NoTrp = TAA vs. No-Trp.

Table 6. Effect of AA infusions on mammary plasma flow (MPF) and mammary AA uptake (mmol/h)

Item	Treatment ¹					SEM	P-value ²			
	CTL	No-Phe	No-Thr	No-Trp	TAA		TAA- Ctl	TAA- NoPhe	TAA- NoThr	TAA- NoTrp
MPF, L/h	498	594	796	611	514	58.9	0.99	0.71	<0.01	0.58
EAA										
His	5.8	6.3	6.9	7.1	7.0	0.45	0.11	0.53	0.99	0.99
Ile	16.6	21.4	24.0	23.8	21.9	1.23	0.03	0.98	0.52	0.61
Leu	25.5	34.3	37.9	38.1	34.4	1.83	0.01	1.00	0.37	0.33
Lys	19.7	23.3	27.3	26.7	22.0	3.13	0.95	0.99	0.57	0.65
Met	6.2	6.1	6.7	7.1	6.9	0.42	0.28	0.19	0.94	0.99
Phe	10.0	9.0	11.6	12.0	11.8	0.77	0.06	0.01	0.98	0.99
Thr	12.0	12.9	13.9	13.8	14.2	0.86	0.17	0.55	0.99	0.99
Trp	2.9	3.2	3.2	2.9	3.1	0.57	0.96	0.99	0.99	0.99
Val	21.4	29.3	29.5	28.4	29.0	1.19	<0.01	0.99	0.96	0.98
NEAA										
Ala	13.9	7.1	3.3	8.4	11.0	4.20	0.84	0.65	0.16	0.87
Asn	5.3	4.6	5.0	5.9	6.1	0.64	0.62	0.20	0.40	0.99
Asp	4.7	3.1	5.0	3.5	2.3	0.98	0.26	0.90	0.18	0.73
Cys	2.2	3.2	2.6	1.9	0.9	0.61	0.40	0.08	0.20	0.64
Gln	26.7	23.7	27.9	26.1	29.6	2.69	0.78	0.25	0.95	0.64
Glu	20.6	20.3	22.5	18.9	17.4	2.21	0.32	0.40	0.07	0.84
Gly	4.2	1.9	-2.7	-1.6	1.9	2.17	0.54	1.00	0.09	0.24
Pro	7.1	8.7	7.6	10.5	11.5	1.19	0.06	0.27	0.10	0.91
Ser	6.6	4.8	2.7	8.1	9.6	2.50	0.41	0.11	0.02	0.87
Tyr	8.9	9.5	9.2	10.2	10.6	0.55	0.05	0.22	0.09	0.81
Group-1 AA-N ³	48.2	50.0	54.3	56.5	56.6	3.31	0.11	0.23	0.90	1.00
Group-2 AA-N ⁴	102.2	130.8	145.4	143.6	129.1	8.99	0.14	0.99	0.48	0.57
TEAA-N ⁵	152.8	183.6	204.6	203.1	189.3	11.90	0.11	0.98	0.70	0.76
TNEAA-N ⁶	132.2	115.3	116.5	124.3	142.4	11.74	0.82	0.15	0.17	0.42
BCAA ⁷	63.6	85.0	91.6	89.9	85.2	3.86	<0.01	1.00	0.42	0.66
TAA-N ⁸	283.6	297.4	322.1	325.9	340.4	17.43	0.14	0.30	0.87	0.93

¹CTL = water infusion; No-Phe = all AA infused excluding Phe; No-Thr = all AA infused excluding Thr; No-Trp = all AA infused excluding Trp; TAA = all AA infused.

²P-values: each treatment was compared with TAA using the Dunnett test; TAA-Ctl = TAA vs. CTL; TAA-NoPhe = TAA vs. No-Phe; TAA-NoThr = TAA vs. No-Thr; TAA-NoTrp = TAA vs. No-Trp.

³Group-1 AA-N = His, Met, Phe, Trp, and Tyr.

⁴Group-2 AA-N = Ile, Leu, Lys, and Val.

⁵TEAA-N = total EAA-N.

⁶TNEAA-N = total NEAA-N.

⁷Branched-chain AA = Ile, Leu, and Val.

⁸TAA-N = total AA-N.

the cows; concentrations did not differ between treatments (Table 8). Mammary uptake of acetate, BHB, glucose, and lactate was unaffected by treatment, averaging 609, 115, 434, and 109 mmol/h, respectively.

Glucose Whole-Body Rate of Appearance

Glucose WBRA did not differ between the AA deletion treatments and TAA (Table 9). Treatment had no effect on the ratio of milk lactose output to glucose WBRA, which averaged 0.57. Treatment also had no effect on milk lactose output as a percentage of glucose mammary uptake, or on glucose mammary uptake as a percentage of WBRA.

DISCUSSION

Milk Yield and Composition

Milk yield was 3.5 kg/d less with CTL than with TAA but this difference remained numerical, similar to the 3.4-kg difference reported by Doelman et al. (2015) between their control and EAA treatments. Unfortunately, studies such as these that are designed to detect metabolic differences do not capture differences in the cows' performance. It is interesting, however, that the milk response in the 2 studies was so similar, considering that Doelman et al. (2015) infused only EAA, whereas we infused both EAA and NEAA. Our rate of EAA infusion was lower than theirs: 430 versus 563

Table 7. Effect of AA infusions on mammary AA uptake:milk protein output ratio

AA	Treatment ¹					SEM	P-value ²			
	CTL	No-Phe	No-Thr	No-Trp	TAA		TAA- Ctl	TAA- NoPhe	TAA- NoThr	TAA- NoTrp
EAA										
His	1.06	1.19	1.14	1.11	1.07	0.05	0.99	0.33	0.74	0.96
Ile	1.19	1.56	1.59	1.43	1.32	0.07	0.09	<0.01	<0.01	0.22
Leu	1.10	1.52	1.50	1.39	1.25	0.05	0.02	<0.01	<0.01	0.02
Lys	1.12	1.35	1.41	1.29	1.02	0.13	0.95	0.28	0.18	0.43
Met	1.02	1.03	1.02	0.99	0.97	0.03	0.46	0.44	0.56	0.94
Phe	1.07	0.98	1.13	1.09	1.07	0.02	1.00	0.09	0.06	0.63
Thr	1.04	1.15	1.10	1.01	1.04	0.04	1.00	0.09	0.22	0.86
Trp	1.28	1.43	1.27	1.10	1.16	0.21	0.78	0.20	0.83	0.99
Val	1.21	1.71	1.54	1.38	1.42	0.08	0.10	0.02	0.37	0.97
NEAA										
Ala	1.19	0.59	0.18	0.62	0.77	0.34	0.50	0.95	0.25	0.97
Asn	0.54	0.48	0.47	0.51	0.53	0.04	0.99	0.81	0.63	0.99
Asp	0.55	0.38	0.56	0.36	0.24	0.11	0.19	0.76	0.18	0.83
Cys	1.13	1.73	1.20	0.83	0.51	0.29	0.38	0.04	0.30	0.82
Gln	1.35	1.23	1.30	1.11	1.28	0.13	0.97	0.98	1.00	0.57
Glu	0.80	0.78	0.80	0.62	0.57	0.07	0.01	0.02	0.01	0.79
Gly	0.53	0.27	-0.38	-0.19	0.21	0.27	0.47	0.99	0.08	0.28
Pro	0.36	0.26	0.34	0.25	0.34	0.04	0.26	0.96	0.16	0.98
Ser	0.35	0.26	0.12	0.38	0.41	0.12	0.90	0.26	0.02	0.99
Tyr	0.93	1.02	0.87	0.91	0.93	0.02	1.00	0.02	0.10	0.69
Group-1 AA-N ³	1.05	1.14	1.08	1.05	1.04	0.03	0.99	0.20	0.67	0.99
Group-2 AA-N ⁴	1.13	1.49	1.47	1.35	1.20	0.07	0.78	0.03	0.03	0.31
TEAA-N ⁵	1.12	1.37	1.35	1.26	1.15	0.05	0.92	0.02	0.03	0.34
TNEAA-N ⁶	0.78	0.69	0.62	0.62	0.71	0.05	0.64	0.99	0.44	0.41
BCAA ⁷	1.16	1.59	1.55	1.39	1.32	0.05	<0.01	<0.01	<0.01	0.19
TAA-N ⁸	0.93	1.00	0.96	0.91	0.93	0.04	1.00	0.54	0.97	0.96

¹CTL = water infusion; No-Phe = all AA infused excluding Phe; No-Thr = all AA infused excluding Thr; No-Trp = all AA infused excluding Trp; TAA = all AA infused.

²P-values: each treatment was compared with TAA using the Dunnett test; TAA-Ctl = TAA vs. CTL; TAA-NoPhe = TAA vs. No-Phe; TAA-NoThr = TAA vs. No-Thr; TAA-NoTrp = TAA vs. No-Trp.

³Group-1 AA-N = His, Met, Phe, Trp, and Tyr.

⁴Group-2 AA-N = Ile, Leu, Lys, and Val.

⁵TEAA-N = total EAA-N.

⁶TNEAA-N = total NEAA-N.

⁷Branched-chain AA = Ile, Leu, and Val.

⁸TAA-N = total AA-N.

Table 8. Effect of AA infusions on arterial concentration and mammary uptake of energy-yielding metabolites

Item	Treatment ¹					SEM	P-value ²			
	CTL	No-Phe	No-Thr	No-Trp	TAA		TAA- Ctl	TAA- NoPhe	TAA- NoThr	TAA- NoTrp
Arterial, mM										
Acetate	1.70	1.49	1.34	1.68	1.54	0.12	0.62	0.99	0.40	0.69
BHB	0.54	0.49	0.48	0.53	0.53	0.04	0.99	0.82	0.74	1.00
Glucose	3.32	3.59	3.45	3.48	3.46	0.12	0.56	0.58	0.99	0.99
Lactate	0.68	0.55	0.45	0.37	0.52	0.09	0.45	0.99	0.91	0.48
Uptake, mmol/h										
Acetate	550	590	666	708	531	80.5	0.99	0.85	0.28	0.13
BHB	98	111	130	120	113	15.2	0.79	0.99	0.75	0.98
Glucose	369	400	505	465	432	41.5	0.56	0.92	0.44	0.90
Lactate	108	122	138	44	133	36.0	0.92	0.99	0.99	0.16

¹CTL = water infusion; No-Phe = all AA infused excluding Phe; No-Thr = all AA infused excluding Thr; No-Trp = all AA infused excluding Trp; TAA = all AA infused.

²P-value: each treatment was compared with TAA using the Dunnett test; TAA-Ctl = TAA vs. CTL; TAA-NoPhe = TAA vs. No-Phe; TAA-NoThr = TAA vs. No-Thr; TAA-NoTrp = TAA vs. No-Trp.

g/d, and the CP concentration of their diet was lower than ours: 11.2 versus 12.0%. Supply of EAA seems, however, to be the driving force for the milk response, as the addition of NEAA to an EAA infusion did not significantly improve milk yield (Doepel and Lapierre, 2010). Metcalf et al. (1996) reported a similar milk response to intravascular administration of EAA or a total AA mixture.

The efficiency of MP utilization for milk production is higher when MP supply is limited (Doepel et al., 2004; Metcalf et al., 2008). Indeed, MP-allowable milk yield, estimated by NRC (2001) using a fixed efficiency of 67%, underestimated milk yield at low MP supply by 11.1 kg for the CTL treatment.

The increase of 133 g of milk protein yield with the TAA treatment compared with CTL resulted in a marginal efficiency of 17%, which is slighter lower than the average marginal recovery of 21% for casein infusion studies reported by Hanigan et al. (1998). If we calculate the amount of infused EAA relative to the milk protein response, then the efficiency is 31%, the same as that of Doelman et al. (2015).

The effect of the Phe deficiency was particularly evident in the milk protein yield response, with the reduction in milk protein yield being similar between No-Phe and CTL. Similarly, Doelman et al. (2015) reported that milk protein yield decreased with the deletion of Phe from an AA mixture (94 g of Phe/d removed from 563 g/d of total EAA supply). Milk protein concentration increased when Phe was added to an EAA mixture (430 g/d excluding Phe) but was not different among the supplemented treatments (supplemented at 37, 21, and 6 g/d; Rulquin and Pisulewski, 2000). This last dose-response study indicates that a low supply of Phe from diets providing between 70 and 75% of MP requirement penalizes milk protein yield but that the full dose does not need to be administered for milk protein yield to be restored.

In the current study, deletion of Thr numerically decreased milk protein yield by only half of the decrease

observed with Phe. In the literature, the effects of changes in Thr supply have not been consistent; addition of Thr to an AA infusion increased milk yield, but addition of Phe and Thr, or Phe, Thr, and His to an AA mixture did not increase milk or milk protein yields (Schwab et al., 1976). It must be noted that, in these studies, the base mixture of AA was not consistent among the trials, which may have affected the response to Phe and Thr. Fraser et al. (1991) and Rulquin and Pisulewski (2006) reported that Thr supplementation did not increase milk and milk protein yields and, in fact, Thr supplementation at a rate of 49 g/d has been reported to decrease true milk protein yield (Rulquin and Pisulewski, 2006). More recently, Appuhamy et al. (2012) reported that casein-rich protein precipitates were lower from mammary tissue slices incubated in EAA-supplemented medium devoid of Thr than from slices incubated with medium containing Thr. Those results, however, were obtained in the absence of Thr, which is obviously not possible to achieve in vivo.

Tryptophan deletion from the infusate had no effect on milk protein yield, as also reported by Doelman et al. (2015), suggesting that even with its deletion, it was supplied in sufficient amounts to meet the cows' needs at low protein supply. Similarly, addition of Met and Trp to an abomasal infusion of soy protein isolate did not increase milk and milk protein yields (Choung and Chamberlain, 1992).

Milk lactose concentration did not change with AA deletions relative to TAA. This result is in contrast to the study of Doepel and Lapierre (2010), in which removal of EAA from an AA abomasal infusion increased milk lactose percentage; however, the result is in agreement with Weekes et al. (2006) where deletion of His, Lys, Met, or the BCAA numerically increased lactose concentration, and with Doelman et al. (2015), who also observed only numerical increases in milk lactose concentration with deletions of EAA, His, Met, or Phe but not Trp from an AA infusion mixture. In the current study, lactose yield was unaffected by deletion of

Table 9. Effect of AA infusions on glucose whole-body rate of appearance (WBRA) and glucose-related ratios

Item	Treatment ¹						P-value ²			
	CTL	No-Phe	No-Thr	No-Trp	TAA	SEM	TAA- Ctl	TAA- NoPhe	TAA- NoThr	TAA- NoTrp
Glucose WBRA, mmol/h	594	636	610	593	645	30.1	0.28	0.99	0.54	0.26
Lactose:glucose WBRA, %	56	55	56	61	58	4.1	0.94	0.89	0.98	0.96
Lactose:glucose mammary uptake, %	89	79	69	81	85	6.5	0.97	0.90	0.24	0.95
Glucose mammary uptake:WBRA, %	64	68	82	80	69	7.9	0.95	1.00	0.47	0.55

¹CTL = water infusion; No-Phe = all AA infused excluding Phe; No-Thr = all AA infused excluding Thr; No-Trp = all AA infused excluding Trp; TAA = all AA infused.

²P-value: each treatment was compared with TAA using the Dunnett test; TAA-Ctl = TAA vs. CTL; TAA-NoPhe = TAA vs. No-Phe; TAA-NoThr = TAA vs. No-Thr; TAA-NoTrp = TAA vs. No-Trp.

individual AA, in agreement with Doelman et al. (2015) and with Haque et al. (2013), in which Arg, Ile, or Val was deleted from an AA infusion. Consistent with that found for the individual AA deletions, lactose yield was not different between CTL and TAA. Although these observations seem not to agree with a review (Lapierre et al., 2010) showing that postruminal infusions of casein or AA with a casein profile consistently resulted in a positive milk lactose yield response, the magnitude of the increment observed in the current study is in the high range of responses. This might indicate that we should carefully interpret the nonsignificance on animal performance observed with the type of study used here, due to the limited number of animals that can be used in such studies.

Urea and AA Metabolism: Arterial Concentrations

Arterial plasma urea-N concentration was drastically decreased with CTL compared with TAA, indicating a deficiency of AA for milk protein synthesis. With No-Phe, plasma urea-N concentration tended to be higher than with TAA, in line with the lower milk protein yield; therefore, less of the AA supply was used to support protein synthesis and the greater excess of AA could be oxidized. There is no clear explanation to reconcile the differences in response between plasma urea-N and MUN for the No-Phe treatment. Weekes et al. (2006) also reported an increase in plasma urea-N concentration and a reduction in milk protein yield when individual AA were removed from a complete AA infusion. Urea-N concentration of No-Thr and No-Trp did not differ from TAA, in line with the lack of effect on milk protein yield with those treatments.

The decrease, or tendency for a decrease, in many EAA arterial concentrations with CTL indicates that the EAA were deficient relative to the cows' requirements; this was expected considering that the diet was formulated to meet only 66% of the cows' MP requirement. Concentration of His showed the greatest decrease of all EAA, with the His concentration being 64% lower in CTL than in TAA, similar to previous observations when CP of the diet was decreased (e.g., Doepel and Lapierre, 2010; Lee et al., 2012). This might be attributed to a greater contribution of microbial protein to MP supply at low CP compared with high CP, acknowledging the low proportion of His in microbial protein (NRC, 2001). Otherwise, with the individual AA deletion treatments, the only EAA that decreased in concentration were those that were deleted in the treatments, decreasing by 60, 76 and 69% for Phe, Thr, and Trp, respectively, relative to TAA. A similar response was observed by Doelman et al. (2015). It is interesting

to note that the low concentration reached for a single AA when deleted from the infusate is lower than the concentration observed when no AA were infused.

The large increase in the arterial concentration of Pro observed in TAA compared with CTL agrees with observations of other studies in which all AA with a casein profile were included in the infusate or when casein was infused (e.g., Guinard and Rulquin, 1994; Doepel and Lapierre, 2010), and is probably related to the high concentration of Pro in casein (10.3% in the current infusion) compared, for example, with microbial protein (3.6%; Clark et al., 1992). The effect on Tyr is unique compared with other free AA infusion studies as usually the infusate does not contain Tyr but is substituted by Phe due to solubility problems (e.g., Doepel and Lapierre, 2010). With the individual AA deletion treatments, the NEAA arterial concentrations were, for the most part, unaffected by treatment.

Mammary Plasma Flow and AA Uptake

With No-Phe, the decrease in milk protein yield was accompanied by a decrease in Phe mammary uptake; the reduction in arterial concentration was only partially compensated for by the numerical increase (16%) in MPF. In contrast, with No-Thr, with which there was no reduction in milk protein yield, MPF increased by 55% relative to TAA. Therefore, despite the lower arterial concentration and AVdiff ($P < 0.01$), there was no significant change in Thr uptake, or indeed, uptake of any of the EAA. The large increase in MPF with No-Thr compared with TAA is likely a reflection of the mammary gland trying to maintain Thr uptake to support protein synthesis in the face of a low arterial Thr concentration. With the No-Thr treatment, Thr arterial concentration was only 24% of that with TAA, whereas with CTL, in which no AA were infused, Thr concentration was 68% of that in TAA. This increase in MPF with a large deficit of a single AA is consistent with some AA deletion studies. Bequette et al. (2000) reported a 33% increase in MPF in goats when a His deficiency was imposed, and Cant et al. (2003) proposed that removal of individual AA would increase MPF. However, it has also been reported that His (Lapierre and Ouellet, 2015) or Lys (Lapierre et al., 2009) deletion from an AA mixture does not alter MPF. The fact that MPF did not increase with No-Phe and No-Trp relative to TAA suggests that other factors are involved in the regulation. One could speculate that the amount of Phe and Thr relative to total digestible EAA supply could influence MPF but that does not seem to be the case in the current study because digestible Phe supply with No-Phe was 61% (6.9 vs. 11.4% of total EAA)

of the requirement predicted by Doepel et al. (2004), whereas the supply of Thr with No-Thr was 67% (7 vs. 10.4%) of the predicted requirement. However, when comparing CTL with TAA, the mammary venous concentration was lower only for Thr and not for Phe or Trp (data not shown), which might indicate a larger deficiency from the basal diet for this AA. Interestingly, an overall reduction in AA availability, as seen with CTL, did not elicit an increment in MPF. Globally, MPF has been reported to decrease linearly with increased MP supply (review: Lapierre et al., 2012), but this effect was not consistent through all the studies included in the review.

The reduced milk protein yield observed in CTL compared with TAA was accompanied only by a numerical decrease in Group 1 AA-N mammary uptake; however, the fact that the U:O ratio of Group 1 AA-N was not different between these 2 treatments indicates a parallel change in the uptake of Group 1 AA-N and milk protein yield. A similar pattern was observed for the No-Phe treatment. The uptake of Tyr was not affected by Phe deletion and therefore, the U:O ratio of Tyr was higher when Phe was deleted, the only treatment where this ratio was greater than 1. A major difference between the CTL and No-Phe treatment is the behavior of the BCAA; the decreased milk protein was accompanied by a decreased uptake for CTL but not for the No-Phe. As a result, the U:O ratio of the BCAA decreased in CTL but increased in No-Phe, as it also did in No-Thr. This would indicate that the mammary gland is an important site of catabolism of the BCAA when they are provided in excess of requirements. Lysine followed a similar pattern but with a smaller magnitude. Interestingly, Thr, for which a clear pattern of mammary metabolism could not be identified (Lapierre et al., 2012), had an increased U:O ratio when supplied in excess with the No-Phe treatment. With No-Trp, we detected no differences in AA uptake relative to TAA, which coincides with the lack of effect on milk and milk protein yield with this treatment.

Energy-Yielding Metabolites

Arterial plasma concentrations of energy-yielding metabolites did not decrease with changes in AA supply, consistent with previous reports (Doepel and Lapierre, 2010, 2011; Doelman et al., 2015). Glucose mammary uptake was not different between the treatments, coinciding with the lack of an effect on lactose yield. Uptakes of acetate and BHB were not different among treatments, in line with the milk fat concentration that was also not affected by treatment. Energy-corrected milk yield was not different between the AA

deletion treatments and TAA. In agreement with this finding, there was no reduction in mammary uptake of BHB, glucose, lactate, or the sum of these nutrients (data not shown) representing total energy uptake by the mammary gland. Doepel and Lapierre (2010) found that mammary uptake of BHB and glucose was unaffected by EAA supply, which agrees with the current work. Treatment had no effect on the ratio of lactose milk output to glucose mammary uptake. Lemosquet et al. (2010), in a meta-analysis of 10 studies, showed that there was no clear relationship between lactose yield and glucose mammary uptake in response to an increase in protein supply, with lactose to glucose uptake ratios ranging from 0.49 to 1.03.

Whole-Body Glucose Rate of Appearance

Glucose WBRa was not different between the AA deletion treatments and TAA. This is not surprising, considering that milk lactose yield and content, and glucose arterial concentration and mammary uptake were unaffected by treatment. This contrasts the study of Lemosquet et al. (2009) in which WBRa increased with casein infusion, concurrent with an increase in milk lactose yield, as usually reported (Lapierre et al., 2010).

It is possible that WBRa is driven as much by glucose precursor availability as it is by metabolic demand, especially for AA. Indeed, when comparing isoenergetic infusions of glucose or glucose precursors, the WBRa increment observed with a NEAA infusion was only 50% of that observed with a glucose infusion (Lemosquet et al., 2009). On the other hand, although no research has been conducted to determine the WBRa changes with a limited supply of individual AA in dairy cows, Weekes et al. (2006) observed an increment in glucose plasma concentrations when His, Lys, or Met was deleted from an AA infusion mixture with a corresponding decrease in milk protein yield.

CONCLUSIONS

Milk protein yield was negatively affected by the deletion of Phe from a total AA infusion but was not affected by deletion of Thr or Trp. Mammary plasma flow increased significantly by 55% with No-Thr in an attempt to maintain mammary supply and uptake of Thr in the face of a massive decline in arterial concentration. Deletion of Trp did not affect milk or milk protein synthesis, in agreement with the lack of effect on AA mammary metabolism. Milk lactose yield was not influenced by the deletion of single AA, consistent with the lack of effect of treatment on mammary glucose

uptake. Overall, the study indicated that a deficiency of Phe negatively affected cow productivity and mammary metabolism but Thr and Trp did not.

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