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Essential amino acid profile of supplemental metabolizable protein affects mammary gland metabolism and whole-body glucose kinetics in dairy cattle

K. Nichols,[1]* J. Dijkstra,[1] M. J. H. Breuer,[1] S. Lemosquet,[2] W. J. J. Gerrits,[1] and A. Bannink[3]
[1]Animal Nutrition Group, Wageningen University and Research, PO Box 338, 6700 AH Wageningen, the Netherlands
[2]PEGASE, INRAE, Institut Agro, 35590 Saint Gilles, France
[3]Wageningen Livestock Research, Wageningen University and Research, PO Box 338, 6700 AH Wageningen, the Netherlands

ABSTRACT

This study investigated mammary gland metabolism and whole-body (WB) rate of appearance (Ra) of glucose in dairy cattle in response to a constant supplemental level of metabolizable protein (MP) composed of different essential AA (EAA) profiles. Five multiparous rumen-fistulated Holstein-Friesian dairy cows (2.8 ± 0.4 lactations; 81 ± 11 d in milk; mean ± standard deviation) were abomasally infused according to a 5 × 5 Latin square design with saline (SAL) or 562 g/d of EAA delivered in different profiles where individual AA content corresponded to their relative content in casein. The profiles consisted of (1) a complete EAA mixture (EAAC), (2) Ile, Leu, and Val (ILV), (3) His, Ile, Leu, Met, Phe, Trp, Val (GR1+ILV), and (4) Arg, His, Lys, Met, Phe, Thr, Trp (GR1+ALT). A total mixed ration (58% corn silage, 16% alfalfa hay, and 26% concentrate on a dry matter basis) was formulated to meet 100 and 83% of net energy and MP requirements, respectively, and was fed at 90% of ad libitum intake on an individual cow basis. Each experimental period consisted of 5 d of continuous abomasal infusion followed by 2 d of no infusion. Arterial and venous blood samples were collected on d 4 of each period for determination of mammary gland AA and glucose metabolism. On d 5 of each period, D-[U-^{13}C]glucose (13 mmol priming dose; continuous 3.5 mmol/h for 520 min) was infused into a jugular vein and arterial blood samples were collected before and during infusion to determine WB Ra of glucose. Milk protein yield did not differ between EAAC, GR1+ILV, and GR1+ALT, or between SAL and ILV, and increased over SAL and ILV with EAAC and GR1+ILV. Mammary plasma flow increased with ILV infusion compared with EAAC and GR1+ILV. Infusion of EAAC tended to increase mammary gland net uptake of total EAA and decreased the mammary uptake to milk protein output ratio (U:O) of non-EAA compared with SAL. Infusion of ILV increased mammary net uptake and U:O of Ile, Leu, and Val markedly over all treatments. The U:O of total Ile, Leu, and Val increased numerically (25%) with GR1+ILV infusion compared with EAAC, and the U:O of total Arg, Lys, and Thr tended to decrease, primarily from decreased U:O of Lys. During GR1+ALT infusion, U:O of total Arg, Lys, and Thr was greater than that during EAAC infusion, whereas U:O of Ile, Leu, and Val did not differ from EAAC. Glucose WB Ra increased 16% with GR1+ALT over SAL, and increased numerically 8 and 12% over SAL with EAAC and GR1+ILV, respectively. The average proportion of lactose yield relative to glucose WB Ra did not differ across treatments and averaged 0.53. On average, 28% of milk galactose arose from nonglucose precursors, regardless of treatment. In conclusion, intramammary catabolism of group 2 AA increased to support milk component synthesis when the EAA profile of MP was incomplete with respect to casein. Further, WB and mammary gland glucose metabolism was flexible in support of milk component synthesis, regardless of absorptive EAA profile.
Key words: essential amino acid, glucose metabolism, protein synthesis, galactose, mammary plasma flow

INTRODUCTION

Mammary gland uptake of AA for milk protein synthesis plays a key role in the efficiency of transfer of dietary N into milk N (Cant et al., 2003; Haque et al., 2015). In order for mammary EAA uptake to be increased, accompanying responses in milk protein synthesis, tissue protein accretion, or intramammary AA catabolism must occur (Cant et al., 2018). Of the EAA, mammary gland net transfer of group 1 AA (His, Met, Phe+Tyr, and Trp) into milk canonically occurs in a 1:1 ratio with their uptake from arterial circulation. A defining characteristic of group 2 AA (Arg, Ile, Leu, Lys, Thr, and Val) is their excess mammary net uptake relative to their output in milk protein (Mepham, 1982; Lapierre et al., 2012). Intramammary metabolism of the excess group 2 AA, particularly the branched-chain

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*Corresponding author: kelly.nichols@wur.nl

AA (Ile, Leu, and Val), Arg, and Lys, provides substantial N and carbon for de novo NEAA synthesis, and for glycolytic and tricarboxylic acid cycle intermediates (Bequette et al., 1996; Mabjeesh et al., 2000; Lapierre et al., 2009). The mammary gland may adapt to deficiencies of single EAA by altering the rate of blood flow to the tissue (Bequette et al., 1996, 2000; Doepel et al., 2016), cellular AA transporter activity (Baumrucker, 1985; Maas et al., 1998; Bequette et al., 2000), the level of intramammary AA catabolism (Lapierre et al., 2009), the rate of protein synthetic activities (Doelman et al., 2015a,b), or through a combination of these, in an effort to maintain milk protein synthesis.

The liver removes AA in coordination with maintenance of whole-body (**WB**) AA homeostasis, substrate supply for gluconeogenesis, AA requirements for constituent and export proteins, and AA requirements of peripheral tissues (Omphalius et al., 2020). Of the EAA, His, Met, and Phe are susceptible to hepatic sequestration that usually increases with increasing supply, whereas liver extraction of Ile, Leu, and Val is negligible across a range of MP supplies (Raggio et al., 2004; Omphalius et al., 2020). Propionate is recognized as the major glucose precursor in fed lactating ruminants, but absorbed AA (except Leu and Lys) can make net contributions to hepatic gluconeogenesis, ranging from 2 to 40% (Danfær et al., 1995; Huntington et al., 2006). Whole-body glucose flux generally increases in response to increased protein supply (Clark et al., 1977; Galindo et al., 2011), but the magnitude of contribution of total AA to glucose production varies, mainly due to physiological and nutrient status (i.e., lactation stage, and the balance in energy and protein supply relative to animal requirements; Danfær et al., 1995).

Apparent differences in AA metabolism by splanchnic and peripheral tissues suggests that the AA profile of MP is important when aiming to improve the transfer of dietary N into milk N. In primary mammary epithelial cells, Yoder et al. (2019) described the capability of several EAA profiles to stimulate protein translation pathways in mammary cells, suggesting the search for a single stimulating profile is moot. Previous studies have evaluated the effects of EAA deficiencies in duodenal supply on milk protein synthesis, where single AA or groups of AA were subtracted from postruminal infusions of complete AA profiles (Weekes et al., 2006; Doelman et al., 2015a,b), but fewer have examined the effects on mammary gland metabolism (Doepel and Lapierre, 2011; Haque et al., 2015; Doepel et al., 2016) or WB rate of appearance (**Ra**) of glucose (Lemosquet et al., 2009a). Therefore, the first objective of the current experiment was to examine mammary gland responses to incomplete EAA profiles. In this experiment, dairy cows postruminally supplemented with 562 g of EAA/d in profiles where Arg, Lys, and Thr, or Ile, Leu, and Val were absent produced the same level of total milk, protein, fat, and lactose as compared with infusion of a complete EAA profile at the same dose (Nichols et al., 2019a). Infusion of only Ile, Leu, and Val resulted in lower milk protein yield compared with the complete EAA profile, but supported the same level of milk protein production as the negative control (saline), despite lower feed intake. Based on the similar milk protein yield produced with supplementation of a complete EAA profile and those lacking Arg, Lys, and Thr or Ile, Leu, and Val, we hypothesized that intramammary catabolism of those EAA would decrease when they were absent from the infusion, and would increase when they were present. We expected intramammary catabolism of the branched-chain AA to increase with infusion of only Ile, Leu, and Val. A second objective of this experiment was to determine if the EAA profile of supplemented MP affected WB Ra of glucose. We hypothesized that differences in affinity of certain AA groups for hepatic metabolism may affect WB Ra of glucose during infusion of incomplete EAA profiles.

MATERIALS AND METHODS

Milking, Feeding, and Treatment Infusions

The experimental procedures were conducted from August to October 2017 at the animal research facilities of Wageningen University & Research (Wageningen, the Netherlands) under the Dutch Law on Animal Experiments in accordance with European Union Directive 2010/63, and were approved by the Central Committee of Animal Experiments (The Hague, the Netherlands). The experimental design, animal housing, ration composition and preparation, and feed chemical analyses have been described in detail by Nichols et al. (2019a). Briefly, the effects of EAA profiles within a constant supplemented MP level were tested using 5 rumen-fistulated, Holstein-Friesian dairy cows (2.8 ± 0.4 lactations; 81 ± 11 DIM) randomly assigned to a 5 × 5 Latin square design, in which each experimental period consisted of 5 d of continuous abomasal infusion followed by 2 d of no infusion (wash-out period). Cows were housed individually in identical climate respiration chambers [**CRC**; described in detail by van Gastelen et al. (2015)], and were allowed 5 d of adaptation to the CRC environment (after an initial 14-d diet adaptation in tiestalls) before the first experimental period began. Cows were fed a TMR (13% CP on DM basis) consisting of 58% corn silage, 16% alfalfa hay, and 26% concentrate on a DM basis which was formulated to meet 100 and 83% of NE_L and MP requirements (CVB, 2008), respectively, for cows consuming 21 kg of DM/d

and producing 33 kg/d of milk containing 41 g/kg fat and 34 g/kg protein. Daily feed intake for individual cows was restricted to 90% of individual daily ad libitum intake determined during the final 5 d of the tiestall diet adaptation before cows entered the CRC. Fresh feed was allocated twice daily during the entire experiment, with the exception of a 58-h window over d 3 to 5 of each period (from 0530 h on d 3 until 1530 h on d 5), where an automated feeding system dispensed equal portions of feed every 2 h to promote metabolic steady-state conditions in preparation for the blood sampling and isotope infusion protocols described below. Cows had individual and free access to drinking water throughout the entire experiment. Cows were milked twice daily at 0530 and 1530 h. Milk was collected, weighed, and sampled at each milking. Samples were stored at 4°C and analyzed within 4 d for protein, fat, lactose, and urea by mid-infrared spectroscopy (ISO 9622; ISO, 2013).

Infusion lines were placed in the abomasum via the rumen cannula 2 d before the first experimental period and were checked daily for patency and position. Abomasal infusion treatments were 0.9% saline (**SAL**) and 4 different AA profiles (562 g/d of anhydrous AA; Table 1) consisting of (1) a complete EAA mixture (**EAAC**), (2) Ile, Leu, and Val (**ILV**), (3) His, Ile, Leu, Met, Phe, Trp, Val (**GR1+ILV**), and (4) Arg, His, Lys, Met, Phe, Thr, Trp (**GR1+ALT**). Within each AA infusion, EAA were infused in amounts relative to their content in 1 kg of casein, according to Metcalf et al. (1996). Including intake from the restricted feeding level of the basal diet and the infusions, target requirements for NE_L and MP were formulated to be met to 90 and 75%, respectively, for SAL, and 95 and 104%, respectively, for AA infusions. Treatment solutions were prepared in 15-L batches, which were stored at room temperature for no more than 48 h before administration. Treatments were replenished daily and infused via multichannel peristaltic pumps at a rate of 10.4 mL/min to facilitate 120 h of continuous infusion.

Blood Sampling, Isotope Infusion, and Analysis

At 0730, 0930, 1130, 1330, and 1530 h on d 4 of each period, blood samples were collected by venipuncture into 10 mL sodium heparin and potassium EDTA vacutainers (Becton Dickinson) from the coccygeal vessels and from the subcutaneous abdominal vein of each cow. Arteriovenous differences (**AVD**) across the tail are assumed to be negligible and thus samples from the coccygeal vessels are representative of mammary arterial supply (Emery et al., 1965). Samples were collected from the left and right subcutaneous abdominal veins, alternating at each sampling time point, to account for differences between sides. Collection tubes were immediately placed in ice and centrifuged at 3,000 × *g* for 15 min at room temperature. Plasma from each time point was collected and stored at −80°C pending analysis of AA, peptides, and AA metabolites. Plasma for analysis of other metabolites was pooled over sampling time points by cow and period and stored at −20°C until analysis. Plasma AA, peptide, and AA metabolite concentrations were determined using an ultra-performance liquid chromatography-MS system (Waters Acquity Ultra Performance LC system, Waters Corp.) as described by Haque et al. (2012). Plasma concentrations of glucose, BHB, nonesterified fatty acids, triacylglycerol (**TAG**), and urea were analyzed as described by Nichols et al. (2019a).

During the 5-d adaptation period in the CRC, cows were fitted with semi-permanent double-lumen catheters (length, 16 cm; o.d. 2.5 mm; distal lumen, i.d. 1.19 mm, o.d. 1.65 mm; proximal lumen, i.d. 0.84 mm, o.d. 1.27 mm; MultiCath 2, art. no. 157.167; Vygon) in the right jugular vein. Catheters were checked twice daily for patency and flushed with heparinized saline for the duration of the experiment. On d 5 of each period, D-[U-^{13}C]glucose (3.5 mmol/h, 99 atom % ^{13}C; Sigma Aldrich) was continuously infused into the right jugular vein of each cow for 520 min, preceded by a priming dose of 13 mmol. Blood was collected from the coccygeal vessels of each cow into 10 mL sodium heparin vacutainers (Becton Dickinson) by venipuncture at 0500 h to serve as a background sample. The infusion

Table 1. Rate of AA infusion (g/d) into the abomasum of lactating dairy cattle over 5 d

	Treatment[2]				
AA[1]	SAL	EAAC	ILV	GR1+ILV	GR1+ALT
L-Arg	0	39	0	0	64
L-His	0	32	0	47	52
L–Ile	0	57	150	84	0
L-Leu	0	94	245	138	0
L-Lys	0	98	0	0	159
DL–Met	0	27	0	40	44
L-Phe	0	94	0	138	152
L-Thr	0	42	0	0	68
L-Trp	0	14	0	21	23
L-Val	0	64	167	94	0
Total	0	562	562	562	562

[1]Anhydrous.

[2]Abomasal infusion treatments were saline (negative control) and 4 different AA profiles. SAL = 0.9% saline infusion; EAAC = a complete mixture of EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); ILV = mixture of Ile, Leu, and Val; GR1+ILV = mixture of His, Ile, Leu, Met, Phe, Trp, and Val; GR1+ALT = mixture of Arg, His, Lys, Met, Phe, Thr, and Trp. Amino acids within treatments were infused in amounts relative to their content in casein. All AA treatments supplied 562 g of MP/d, and solutions were delivered in 15-L batches.

for individual cows began approximately 15 min after their morning milking was completed. Blood samples were collected from the coccygeal vessels of each cow 460 and 490 min after the infusion started. Collection tubes were immediately placed in ice and centrifuged at 3,000 × *g* for 15 min at room temperature. Plasma from each time point was collected and stored at −80°C. Additional milk samples were collected from each cow during milking on d 5 and stored at −20°C.

Blood plasma (100 μL) was treated with 0.8 mL acetonitrile followed by centrifugation (3,000 × *g* for 10 min at room temperature), and the supernatant was evaporated at 45°C for 40 min under vacuum. Milk samples collected on d 5 were defatted, deproteinized, and hydrolyzed with β-galactosidase (EC 3.2.1.23) using the methods described by Sunehag et al. (2002). Hydrolyzed milk serum (50 μL) was dried under vacuum at 45°C for 2 h. According to the methods described by Schierbeek et al. (2009), aldonitrile pentaacetate derivatives of blood plasma and hydrolyzed milk serum were prepared by adding 100 μL of 2% hydroxylamine-HCl to pyridine and incubating together at 90°C for 30 min. Subsequently, 50 μL acetic anhydride was added and incubated at 90°C for 30 min. Remaining liquid was evaporated under a N stream at 50°C. Samples were solubilized with 100 μL chloroform and analyzed for ^{13}C enrichment (in plasma glucose and in milk glucose and galactose) by GC combustion isotope ratio MS analysis using a DELTA V isotope ratio mass spectrometer (Thermo Fisher Scientific) coupled with a Trace Ultra GC (Thermo Fisher Scientific). A Zebron ZB-MultiResidue-1 capillary column (length, 30 m; i.d. 0.25 mm; film thickness 0.25 μm; Phenomenex) and splitless injection was used for the chromatographic separation. The temperature was held at 60°C for 0.05 min, then raised 14.5°C/min and held at 230°C for 6 min. After separation, the combustion phase used an oven temperature held at 60°C for 2 min, raised 25°C/min, and finally held at 230°C for 11.2 min. The flow of helium carrier gas was maintained at 2.0 mL/min.

Gaseous exchange (CH_4, O_2, and CO_2) in the CRC was measured as described by Nichols et al. (2019a). Concentrations of ^{13}C were analyzed in outgoing air in 10-min intervals by nondispersive infrared spectrometry (Advance Optima Uras 14 NDIR analyzer; ABB) as described by Alferink et al. (2003). The analyzer was faulty in one chamber and could not be repaired during the experiment, resulting in measurement of $^{13}CO_2$ dynamics in 4 of 5 chambers per period.

Calculations and Statistical Analysis

Long-chain fatty acid concentrations were calculated on a molar basis as 3 × TAG + nonesterified fatty acids. Plasma concentrations of AA, peptides, and AA metabolites were averaged over the 5 sampling times on d 4. Milk CP was assumed to consist of 94.5% true protein (DePeters and Ferguson, 1992). All following calculations were based on this estimate of true protein yield. Mammary plasma flow (**MPF**) across the whole udder was estimated according to the Fick principle using Phe and Tyr as internal markers (Cant et al., 1993), where MPF (L/h) = [milk Phe + Tyr output (μmol/h)]/[Phe + Tyr AVD (μmol/L)], with an allowance for 3.37% contribution of blood-derived proteins to milk Phe + Tyr (Lapierre et al., 2012). Milk output of Phe + Tyr was estimated from the afternoon milk protein yield of d 4 of infusion, corresponding to the blood samples taken that day, using mean Phe and Tyr contents reported by Mepham (1987) and Lapierre et al. (2012). Uptakes (mmol/h) of metabolites across the mammary glands were calculated as the product of their plasma AVD and MPF. Positive uptake values indicate a net removal from plasma, whereas negative values indicate net release from the mammary glands. Mammary clearances were calculated from the model of Hanigan et al. (1998), where clearance (L/h) = (AVD × MPF)/venous concentration. The average milk protein AA composition of that reported by Mepham (1987) and Lapierre et al. (2012) and milk protein yield from the afternoon milking on d 4 of infusion was used to calculate mammary gland AA uptake to milk true protein output ratios (**U:O**). Mammary gland glucose balance was calculated according to estimations of Dijkstra et al. (1996) using milk lactose and fat yield from the afternoon milking on d 4 of infusion.

Plasma samples collected after the start of tracer infusion were adjusted for background isotopic enrichment (**IE**) of the 0500 h sample to calculate atom percent excess (**APE**) of ^{13}C in plasma glucose during the tracer infusion. Plasma APE was analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc.) for treatment, sampling time, and treatment × sampling time effects, with treatment and period as fixed effects, cow as a random effect, and sampling time as a repeated measure using a first-order autoregressive covariance structure. There was no treatment × sampling time effect on plasma APE ($P = 0.84$). Multiple comparisons between treatment least squares means for sampling time point determined that APE at 460 and 490 min after initiation of tracer infusion did not differ ($P > 0.65$). Therefore, the animals were considered to be in steady-state after 460 min of infusion, and the mean APE of the 2 samples were used to calculate WB Ra of glucose (mmol/h) using the steady-state model:

$$\text{WB Ra} = \text{F} \times [(\text{IE}_{\text{inf}}/\text{IE}_{\text{p}}) - 1],$$

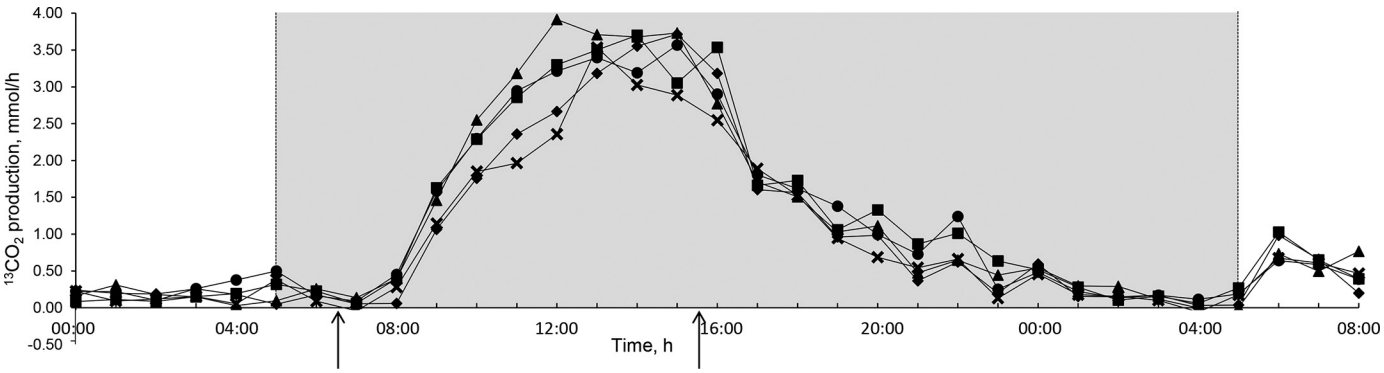

Figure 1. Production of $^{13}CO_2$ (mmol/h) during a primed continuous infusion of D-[U-^{13}C]glucose in lactating dairy cows receiving abomasal infusions of saline (SAL) or 562 g/d of AA in different profiles; SEM averaged 0.320, 0.278, 0.278, 0.320, and 0.278 for SAL (■), EAAC (◆), ILV (▲), GR1+ILV (×), and GR1+ALT (●), respectively. n = 4 for all treatments except GR1+ALT, where n = 3. Arrows indicate the mean time of isotope infusion start and end across cow and period. The shaded area indicates the 24-h period (0500 h on d 5 to 0500 h on d 6) used for calculation of the incremental area under the curve and ^{13}C recovery. SAL = 0.9% saline infusion; EAAC = a complete mixture of EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); ILV = mixture of Ile, Leu, and Val; GR1+ILV = mixture of His, Ile, Leu, Met, Phe, Trp, and Val; GR1+ALT = mixture of Arg, His, Lys, Met, Phe, Thr, and Trp. Amino acids within treatments were infused in amounts relative to their content in casein. All AA treatments supplied 562 g of MP/d, and solutions were delivered in 15-L batches.

where F is the D-[U-^{13}C]glucose infusion rate (3.5 mmol/h), IE_{inf} is the IE of ^{13}C in the infusate (99 atom %), and IE_p is the IE of ^{13}C in plasma glucose. Infusion of U-^{13}C-glucose and measurement of total IE may result in an underestimation of WB Ra if isotopomers m+1, m+2, and m+3 formed during the infusion period are not measured; however, the formation of these glucose isotopomers in dairy cattle is minimal (H. Lapierre, Agriculture and Agri-Food Canada, Sherbrooke, Quebec, personal communication), and thus the quantification of total enrichment in this experiment is considered appropriate.

Hourly IE of $^{13}CO_2$ was calculated as the average of all ^{13}C measurements within each hour. Hourly background IE of $^{13}CO_2$ from d 4 (no tracer infusion) was used to calculate hourly $^{13}CO_2$ production (the product of $^{13}CO_2$ APE and hourly CO_2 production) for a 32-h period (0000 h on d 5 through 0800 h on d 6) covering the period of tracer infusion (Figure 1). The positive incremental area under the curve for $^{13}CO_2$ production (mmol) was evaluated using the trapezoid rule for values above baseline and was calculated for the 24 h period between 0500 h on d 5 and 0500 h on d 6. Production of $^{13}CO_2$ over this 24 h period was used to calculate the recovery of infused ^{13}C glucose in expired CO_2. Assuming all ^{13}C recovered in CO_2 arose from Ra glucose, glucose oxidation rate (mmol/h) was calculated as the product of ^{13}C recovery in CO_2 (as a fraction of infused ^{13}C) and WB Ra of glucose (mmol/h) after applying a correction factor of 0.70 to account for ^{13}C sequestration in bicarbonate (Junghans et al., 2007).

One cow did not receive the correct treatment in period 1 and was thus removed from the statistical analysis for this period (n = 4 for GR1+ALT; n = 5 for all other treatments). The jugular catheter of 1 cow lost patency in period 5 resulting in a lost observation for isotope-related measurements in this period. This cow was housed in the chamber with the faulty $^{13}CO_2$ analyzer, resulting in no additional missing measurements of $^{13}CO_2$ dynamics. Variances in milk yield and composition, plasma constituents, and parameters relating to mammary metabolism and WB glucose metabolism were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc.). The model contained treatment and period as fixed effects and cow as a random effect. We observed no carryover effects between periods, assessed by testing for an effect of the previous treatment in the ANOVA. Differences were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Multiple comparisons between treatment means were made using the Tukey-Kramer method when a treatment effect was detected at $P \leq 0.05$. Tendencies for differences between treatments are described in the Supplemental File S1 (https://data.mendeley.com/datasets/gxtcx8rn3w/1; Nichols, 2022). Mammary clearance results for individual AA are described in Supplemental Table S1 (https://data.mendeley.com/datasets/gxtcx8rn3w/1; Nichols, 2022). Arterial concentrations and mammary metabolism of glucose (beyond what is described below), urea, BHB, nonesterified fatty acids, TAG, and long-chain fatty acids is described in Supplemental Tables S2 and S3 (https://data.mendeley.com/datasets/gxtcx8rn3w/1; Nichols, 2022).

Table 2. Milk and component production of lactating dairy cows receiving 5-d abomasal infusions of saline (SAL) or 562 g/d of AA as a complete mixture of EAA (EAAC), as a mixture of Ile, Leu, and Val (ILV), as a mixture of group 1 AA and Ile, Leu, and Val (GR1+ILV), and as a mixture of group 1 AA and Arg, Lys, and Thr (GR1+ALT)[1]

	Treatment[2]						
Item	SAL	EAAC	ILV	GR1+ILV	GR1+ALT	SEM	*P*-value
Milk, kg/h	1.27	1.49	1.35	1.49	1.42	0.088	0.04
CP, g/h	39.1^{b}	48.5^{a}	39.3^{b}	47.8^{a}	44.6^{ab}	2.30	0.01
CP, g/kg	30.8	32.6	29.4	32.2	31.6	1.05	0.05
Fat, g/h	61.5	61.7	66.8	68.2	62.0	4.03	0.20
Fat, g/kg	48.4^{ab}	41.9^{b}	50.8^{a}	46.3^{ab}	44.9^{ab}	3.76	0.05
Lactose, g/h	60.0	68.6	63.7	67.9	66.9	4.02	0.11
Lactose, g/kg	47.1^{ab}	46.1^{bc}	47.3^{a}	45.6^{c}	47.0^{ab}	0.36	<0.01

$^{a–c}$Means within a row with no common superscripts differ ($P < 0.05$).

[1]Data are least squares means from the afternoon milkings on d 4 and 5 of infusion.

[2]Abomasal infusion treatments were saline (negative control) and 4 different AA profiles. SAL = 0.9% saline infusion; EAAC = a complete mixture of EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); ILV = mixture of Ile, Leu, and Val; GR1+ILV = mixture of His, Ile, Leu, Met, Phe, Trp, and Val; GR1+ALT = mixture of Arg, His, Lys, Met, Phe, Thr, and Trp. Amino acids within treatments were infused in amounts relative to their content in casein. All AA treatments supplied 562 g of MP/d, and solutions were delivered in 15-L batches.

RESULTS

Milk Production

Daily lactation performance and DMI have been presented by Nichols et al. (2019a). Daily DMI averaged (mean ± SD) 19.9 ± 0.28 kg/d for SAL, EAAC, GR1+ILV, and GR1+ALT, and was 17.9 kg/d for ILV. The present paper reports milk production expressed on an hourly basis from the afternoon milking on d 4 and 5 of infusion (Table 2). Milk protein yield increased over SAL with EAAC and GR1+ILV ($P \leq 0.03$). Infusion of ILV decreased milk protein yield compared with EAAC and GR1+ILV ($P \leq 0.04$). Infusion of ILV increased milk fat content over EAAC ($P = 0.03$). Lactose content was lower on GR1+ILV infusion compared with SAL, ILV, and GR1+ALT ($P \leq 0.03$), and lower on EAAC compared with ILV ($P = 0.05$).

Arterial Concentrations

Arterial plasma concentration of EAA, group 1 AA, group 2 AA, and nonbranched (**NB**)-group 2 AA increased with EAAC infusion over SAL ($P \leq 0.05$; Table 3). Individually, concentration of Arg, His, Lys, Met, Phe, and Trp increased over SAL with EAAC ($P \leq 0.02$). Infusion of ILV increased arterial concentration of total AA (**TAA**) over SAL, EAAC, and GR1+ALT ($P \leq 0.03$), and increased concentrations of EAA, group 2 AA, and branched-chain (**BC**)-group 2 AA (as a group and individually) over SAL and the other AA infusions ($P \leq 0.01$). Group 1 AA concentrations (as a group and individually) were lower during ILV infusion compared with the other AA infusions ($P \leq 0.01$), and did not differ from SAL ($P \geq 0.28$). Concentration of NB-group 2 AA (as a group and individually) were lower during ILV infusion compared with EAAC and GR1+ALT ($P \leq 0.01$). Infusion of GR1+ILV increased arterial concentration of TAA, EAA, group 1 AA, group 2 AA, and BC-group 2 AA over SAL ($P \leq 0.02$), and increased group 1 AA concentration and decreased NB-group 2 AA concentrations (as a group and individually) compared with EAAC ($P < 0.01$). Infusion of GR1+ALT increased group 1 AA and NB-group 2 AA concentrations over SAL and EAAC ($P < 0.01$). In response to GR1+ILV and GR1+ALT, individual concentrations of all EAA included in the respective infusions increased over SAL ($P \leq 0.01$), with the exception of Ile during GR1+ILV infusion. Infusion of GR1+ILV increased concentrations of His, Met, and Phe compared with EAAC ($P \leq 0.02$). Infusion of GR1+ALT increased concentrations of Lys, Met, Phe, Thr, and Trp ($P \leq 0.04$) and decreased the concentration of Val ($P = 0.04$) compared with EAAC. Concentration of BC-group 2 AA (as a group), Leu, and Val increased over GR1+ALT with GR1+ILV infusion ($P < 0.01$), and concentration of NB-group 2 AA (as a group and individually) increased over GR1+ILV with GR1+ALT infusion ($P < 0.01$).

Infusion of EAAC increased arterial concentration of Orn ($P < 0.01$) and decreased concentration of Ser ($P = 0.02$) compared with SAL. Infusion of ILV increased concentration of Cit ($P = 0.04$) and decreased concentrations of Ala and Tyr ($P \leq 0.04$) compared with SAL. In response to ILV, concentration of Cys and Tyr decreased compared with all other AA infusions

Table 3. Arterial plasma concentrations (μM) of AA, peptides, and AA metabolites in lactating dairy cows receiving 5-d abomasal infusions of saline (SAL) or 562 g/d of AA as a complete mixture of EAA (EAAC), as a mixture of Ile, Leu, and Val (ILV), as a mixture of group 1 AA and Ile, Leu, and Val (GR1+ILV), and as a mixture of group 1 AA and Arg, Lys, and Thr (GR1+ALT)

	Treatment[1]						
Item	SAL	EAAC	ILV	GR1+ILV	GR1+ALT	SEM	*P*-value
EAA[2]	670^{c}	1,351^{b}	2,174^{a}	1,545^{b}	1,117^{bc}	147.8	<0.01
Group 1[3]	147^{c}	274^{b}	116^{c}	430^{a}	409^{a}	12.2	<0.01
Group 2[4]	556^{c}	1,111^{b}	2,080^{a}	1,182^{b}	772^{bc}	146.1	<0.01
BC-Group 2[5]	354^{c}	753^{bc}	1,933^{a}	1,021^{b}	234^{c}	141.2	<0.01
NB-Group 2[6]	202^{c}	358^{b}	148^{c}	161^{c}	539^{a}	24.6	<0.01
NEAA[7]	1,160	948	1,054	1,133	1,199	58.1	0.07
TAA[8]	1,831^{c}	2,299^{bc}	3,228^{a}	2,678^{ab}	2,352^{bc}	169.4	<0.01
Arg	55^{b}	90^{a}	50^{b}	55^{b}	118^{a}	6.8	<0.01
His	23^{c}	60^{b}	20^{c}	83^{a}	75^{ab}	5.0	<0.01
Ile	106^{b}	169^{b}	363^{a}	207^{b}	93^{b}	30.4	<0.01
Leu	89^{c}	206^{bc}	588^{a}	297^{b}	60^{c}	44.2	<0.01
Lys	54^{c}	116^{b}	37^{c}	48^{c}	175^{a}	9.1	<0.01
Met	17^{c}	48^{b}	11^{c}	91^{a}	77^{a}	3.1	<0.01
Phe	46^{c}	94^{b}	36^{c}	145^{a}	145^{a}	8.6	<0.01
Thr	93^{bc}	151^{b}	61^{c}	58^{c}	245^{a}	13.8	<0.01
Trp	29^{c}	38^{b}	27^{c}	45^{ab}	47^{a}	2.4	<0.01
Val	160^{cd}	378^{bc}	982^{a}	517^{b}	105^{d}	68.2	<0.01
Ala	220^{a}	162^{ab}	128^{b}	196^{a}	205^{a}	16.3	<0.01
Asn	42	36	40	43	42	2.9	0.51
Asp	15^{ab}	13^{ab}	11^{b}	16^{a}	15^{ab}	1.0	0.02
Cit	57^{b}	75^{ab}	88^{a}	84^{ab}	68^{ab}	9.3	0.05
Cys	3.9^{ab}	6.1^{a}	2.7^{b}	5.9^{a}	6.6^{a}	0.63	0.01
Gln	251^{ab}	183^{b}	289^{a}	222^{ab}	208^{ab}	18.0	0.01
Glu	38	33	33	32	37	3.1	0.32
Gly	311	241	270	278	313	22.8	0.11
Orn	25^{c}	41^{b}	22^{c}	26^{c}	62^{a}	2.5	<0.01
Pro	69	60	55	74	75	4.9	0.05
Ser	94^{a}	65^{b}	94^{a}	90^{a}	101^{a}	6.3	0.01
Tyr	33^{b}	34^{b}	22^{c}	67^{a}	66^{a}	2.6	<0.01
Other AA, peptides, and AA metabolites[9]							
1 Methyl-histidine	3.8^{a}	3.0^{ab}	2.4^{b}	2.9^{b}	2.7^{b}	0.34	<0.01
3 Methyl-histidine	3.9	3.0	3.0	3.8	2.7	0.35	0.19
α-Amino-*n*-butyric acid	11^{c}	22^{ab}	13^{bc}	22^{ab}	32^{a}	2.5	<0.01
α-Amino-adipic acid	2.9^{c}	6.0^{b}	1.8^{c}	1.2^{c}	9.1^{a}	0.54	<0.01
β-Alanine	3.7	3.8	3.8	3.9	2.9	0.29	0.20
Carnosine	9	11	9	12	12	1.1	0.05
Cystathionine	1.7^{cd}	2.3^{c}	0.6^{d}	3.8^{b}	5.3^{a}	0.31	<0.01
Hydroxylysine	0.48	0.44	0.27	0.44	0.40	0.085	0.37
Hydroxyproline	11^{ab}	10^{ab}	8^{b}	12^{a}	10^{ab}	0.9	0.03
Phosphoserine	0.52	0.57	0.82	0.77	0.58	0.081	0.07
Taurine	29^{b}	29^{b}	26^{b}	45^{a}	50^{a}	3.2	<0.01

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

[1]Abomasal infusion treatments were saline (negative control) and 4 different AA profiles. SAL = 0.9% saline infusion; EAAC = a complete mixture of EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); ILV = mixture of Ile, Leu, and Val; GR1+ILV = mixture of His, Ile, Leu, Met, Phe, Trp, and Val; GR1+ALT = mixture of Arg, His, Lys, Met, Phe, Thr, and Trp. Amino acids within treatments were infused in amounts relative to their content in casein. All AA treatments supplied 562 g of MP/d, and solutions were delivered in 15-L batches.

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

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

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

[5]BC-Group 2 = Ile, Leu, Val.

[6]NB-Group 2 = Arg, Lys, Thr.

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

[8]TAA = EAA + NEAA.

[9]Other N derivatives measured with ultra-performance liquid chromatography-mass spectrometry showing a plasma concentration higher than the limit of quantification.

($P \leq 0.03$), Ser concentration increased over EAAC ($P = 0.01$), Ala and Asp concentration decreased compared with GR1+ILV ($P \leq 0.03$), and Ala concentration decreased compared with GR1+ALT ($P = 0.02$). Concentration of Gln increased with ILV over EAAC ($P = 0.01$) and concentration of Orn decreased with

ILV compared with EAAC and GR1+ALT ($P < 0.01$). Compared with SAL, infusion of GR1+ILV increased concentration of Tyr ($P < 0.01$) and GR1+ALT infusion increased concentration of Orn and Tyr ($P < 0.01$). Compared with EAAC, concentrations of Ser and Tyr increased with GR1+ILV and GR1+ALT ($P \leq 0.04$), and concentration of Orn decreased with GR1+ILV and increased with GR1+ALT ($P \leq 0.01$). Concentration of Orn increased over GR1+ILV with infusion of GR1+ALT ($P < 0.01$).

Arterial plasma concentration of 1 methyl-histidine decreased with ILV, GR1+ILV, and GR1+ALT ($P \leq 0.03$) compared with SAL. Concentration of α-amino-*n*-butyric acid increased over SAL with EAAC, GR1+ILV and GR1+ALT infusions ($P \leq 0.05$), and increased over ILV ($P < 0.01$) with GR1+ALT infusion. Concentration of α-amino-adipic acid increased over SAL, ILV, and GR1+ILV with infusion of EAAC and GR1+ALT ($P \leq 0.01$), and increased over EAAC with GR1+ALT infusion ($P = 0.01$). Cystathionine concentration decreased with ILV infusion compared with all other AA infusions ($P \leq 0.01$), increased with GR1+ILV and GR1+ALT infusions over SAL and EAAC ($P \leq 0.02$), and increased with GR1+ALT infusion compared with GR1+ILV ($P = 0.03$). Infusion of ILV decreased hydroxyproline concentration compared with GR1+ILV ($P = 0.02$). Infusion of GR1+ILV and GR1+ALT increased concentration of taurine over SAL, EAAC, and ILV ($P \leq 0.02$).

Mammary Arteriovenous Differences

Compared with SAL, infusion of EAAC increased AVD of EAA, group 2 AA, BC-group 2 AA, and NB-group 2 AA ($P \leq 0.02$; Table 4). Individual AVD of His, Ile, Leu, and Lys increased ($P \leq 0.04$) with EAAC over SAL. Infusion of ILV increased AVD of group 2 AA ($P = 0.05$) and BC-group 2 AA (as a group and individually; $P < 0.05$), and decreased AVD of Lys ($P = 0.01$) compared with SAL. Arteriovenous difference of NB-group 2 AA decreased with ILV compared with SAL and all AA infusions ($P \leq 0.03$). Compared with all AA infusions, ILV decreased AVD of group 1 AA, and individual AVD of Arg, His, Lys, Met, and Thr ($P \leq 0.04$). Infusion of ILV increased AVD of BC-group 2 AA (as a group and individually) compared with GR1+ALT ($P \leq 0.02$), and increased AVD of Val compared with EAAC ($P = 0.02$). Infusion of GR1+ILV increased AVD of EAA, group 2 AA, and BC-group 2 AA ($P < 0.01$) compared with SAL. Individual AVD of His, Ile, Leu, Phe, and Val increased with GR1+ILV ($P \leq 0.03$) compared with SAL. Compared with EAAC, infusion of GR1+ILV increased AVD of BC-group 2 AA (as a group and individually; $P \leq 0.04$) and decreased AVD of NB-group 2 AA and Lys ($P \leq 0.05$). Infusion of GR1+ALT increased AVD of NB-group 2 AA and Lys compared with SAL ($P < 0.01$). Arteriovenous difference of BC-group 2 AA (as a group and individually) increased over GR1+ALT with GR1+ILV infusion ($P \leq 0.01$), and AVD of NB-group 2 AA and Lys increased over GR1+ILV with GR1+ALT infusion ($P < 0.01$).

Infusion of ILV decreased AVD of Pro ($P = 0.03$) compared with SAL, decreased AVD of Asn and Gln compared with EAAC and GR1+ILV ($P \leq 0.05$), and decreased AVD of Asn compared with GR1+ALT ($P = 0.02$). Further, infusion of ILV decreased AVD of Tyr compared with EAAC and GR1+ILV ($P \leq 0.02$), and decreased AVD of Cys compared with GR1+ILV ($P = 0.04$). Infusion of GR1+ILV decreased AVD of Ala ($P = 0.03$) and increased AVD of Cys ($P = 0.03$) compared with SAL. Infusion of GR1+ALT increased AVD of Orn over SAL, ILV, and GR1+ILV ($P \leq 0.02$).

Mammary Plasma Flow and AA Uptake

Mammary plasma flow was faster on ILV infusion compared with EAAC and GR1+ILV ($P \leq 0.05$; Table 5). Infusion of EAAC increased uptake of Leu and Phe compared with SAL ($P \leq 0.04$). Infusion of ILV increased mammary uptake of EAA, group 2 AA, and BC-group 2 AA (as a group and individually) over SAL ($P < 0.01$). Infusion of ILV increased uptake of BC-group 2 AA, Leu, and Val over all AA infusions ($P \leq 0.03$), increased uptake of Ile and decreased uptake of Lys compared with EAAC and GR1+ALT ($P \leq 0.01$), decreased Phe uptake compared with EAAC and GR1+ILV ($P \leq 0.01$), and decreased Thr uptake compared with GR1+ALT ($P = 0.03$). Infusion of GR1+ILV increased mammary uptake of EAA and BC-group 2 AA ($P \leq 0.04$) over SAL. Individual uptakes of Ile, Leu, and Phe increased ($P < 0.01$) with GR1+ILV infusion over SAL. Uptake of Lys decreased ($P = 0.02$) with GR1+ILV compared with EAAC. Infusion of GR1+ALT increased uptake of NB-group 2 AA, Lys, and Thr over SAL ($P \leq 0.04$) and increased uptake of NB-group 2 AA over ILV and GR1+ILV ($P < 0.01$). Uptake of Ile and Leu increased ($P \leq 0.02$) with GR1+ILV over GR1+ALT. Uptake of Lys was higher ($P < 0.01$) on GR1+ALT compared with GR1+ILV. Infusion of EAAC decreased mammary uptake of Ala and Pro ($P \leq 0.04$) compared with SAL. Infusion of ILV decreased uptake of Asn compared with EAAC and GR1+ALT ($P \leq 0.05$), and increased uptake of Cit compared with EAAC ($P = 0.04$). Infusion of GR1+ILV decreased mammary uptake of Ala and Pro and increased uptake of Cys compared with SAL ($P \leq 0.03$).

Table 4. Mammary gland arteriovenous differences (μM) of AA in lactating dairy cows receiving 5-d abomasal infusions of saline (SAL) or 562 g/d of AA as a complete mixture of EAA (EAAC), as a mixture of Ile, Leu, and Val (ILV), as a mixture of group 1 AA and Ile, Leu, and Val (GR1+ILV), and as a mixture of group 1 AA and Arg, Lys, and Thr (GR1+ALT)

	Treatment[1]						
AA	SAL	EAAC	ILV	GR1+ILV	GR1+ALT	SEM	*P*-value
EAA[2]	223[c]	299[ab]	261[bc]	323[a]	277[abc]	16.6	<0.01
Group 1[3]	52[ab]	67[a]	41[b]	68[a]	59[a]	3.8	<0.01
Group 2[4]	185[b]	250[a]	231[a]	270[a]	233[ab]	15.1	<0.01
BC-Group 2[5]	104[d]	144[bc]	170[ab]	183[a]	119[cd]	12.1	<0.01
NB-Group 2[6]	81[b]	106[a]	61[c]	87[b]	113[a]	4.4	<0.01
NEAA[7]	197	176	119	176	173	18.8	0.10
TAA[8]	420	475	380	498	443	30.3	0.08
Arg	25[ab]	31[a]	21[b]	29[a]	31[a]	1.1	<0.01
His	10[bc]	12[a]	8[c]	12[a]	11[ab]	0.5	<0.01
Ile	27[d]	39[bc]	42[ab]	49[a]	32[cd]	3.3	<0.01
Leu	40[d]	59[bc]	64[ab]	72[a]	46[cd]	4.2	<0.01
Lys	35[b]	48[a]	23[c]	34[b]	56[a]	2.6	<0.01
Met	9[ab]	12[a]	7[b]	12[a]	10[a]	0.7	<0.01
Phe	16[bc]	22[ab]	13[c]	23[a]	19[abc]	1.5	<0.01
Thr	22[ab]	27[a]	16[b]	25[a]	27[a]	1.4	<0.01
Trp	3.5	4.2	3.0	4.7	3.7	0.39	0.07
Val	36[b]	46[b]	64[a]	62[a]	41[b]	4.9	<0.01
Ala	34[a]	8[ab]	14[ab]	6[b]	10[ab]	5.8	0.03
Asn	9[ab]	13[a]	5[b]	11[a]	11[a]	1.0	<0.01
Asp	1.6	0.1	0.8	1.2	1.8	0.38	0.06
Cit	1.8	0.3	2.7	2.5	1.5	0.69	0.14
Cys	0.16[b]	0.49[ab]	0.19[b]	0.79[a]	0.33[ab]	0.128	0.03
Gln	54[ab]	59[a]	31[b]	65[a]	58[ab]	6.4	0.02
Glu	28	23	21	22	28	2.5	0.11
Gly	19	13	12	13	12	4.4	0.72
Orn	13[b]	14[ab]	11[b]	13[b]	19[a]	1.4	<0.01
Pro	11[a]	8[ab]	6[b]	8[ab]	8[ab]	0.9	0.05
Ser	11	20	5	16	10	4.4	0.06
Tyr	14[ab]	17[a]	11[b]	16[a]	15[ab]	1.1	0.01

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

[1]Abomasal infusion treatments were saline (negative control) and 4 different AA profiles. SAL = 0.9% saline infusion; EAAC = a complete mixture of EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); ILV = mixture of Ile, Leu, and Val; GR1+ILV = mixture of His, Ile, Leu, Met, Phe, Trp, and Val; GR1+ALT = mixture of Arg, His, Lys, Met, Phe, Thr, and Trp. Amino acids within treatments were infused in amounts relative to their content in casein. All AA treatments supplied 562 g of MP/d, and solutions were delivered in 15-L batches.

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

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

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

[5]BC-Group 2 = Ile, Leu, Val.

[6]NB-Group 2 = Arg, Lys, Thr.

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

[8]TAA = EAA + NEAA.

Mammary Clearance of AA Groups

Mammary clearance of EAA decreased in response to all AA infusions compared with SAL ($P \leq 0.02$; Table 6). Infusion of EAAC decreased clearance of group 2 AA ($P \leq 0.03$) compared with SAL. Infusion of ILV decreased clearance of TAA, group 2 AA, and BC-group 2 AA ($P \leq 0.02$) compared with SAL. Infusion of ILV increased clearance of group 1 AA ($P < 0.01$) over all other AA infusions, and increased clearance of NB-group 2 AA over EAAC and GR1+ALT ($P \leq 0.01$). Infusion of GR1+ILV decreased mammary clearance of group 1 and group 2 AA ($P \leq 0.02$) and increased clearance of NB-group 2 AA ($P = 0.05$) compared with SAL. Infusion of GR1+ILV increased clearance of NB-group 2 AA over EAAC ($P = 0.02$). Compared with SAL, infusion of GR1+ALT decreased mammary clearance of group 1 AA and NB-group 2 AA ($P \leq 0.05$). Infusion of GR1+ALT increased mammary clearance of BC-group 2 AA over all other AA infusions ($P = 0.01$). Clearance of NB-group 2 AA increased over GR1+ALT with GR1+ILV infusion ($P = 0.01$).

Table 5. Whole-mammary gland plasma flow and net AA uptakes in lactating dairy cows receiving 5-d abomasal infusions of saline (SAL) or 562 g/d of AA as a complete mixture of EAA (EAAC), as a mixture of Ile, Leu, and Val (ILV), as a mixture of group 1 AA and Ile, Leu, and Val (GR1+ILV), and as a mixture of group 1 AA and Arg, Lys, and Thr (GR1+ALT)

	Treatment[1]						
Item	SAL	EAAC	ILV	GR1+ILV	GR1+ALT	SEM	*P*-value
Plasma flow, L/h	768^{ab}	703^{b}	965^{a}	693^{b}	767^{ab}	70.1	0.04
Net mammary uptake, mmol/h							
EAA[2]	167^{b}	209^{ab}	248^{a}	219^{a}	210^{ab}	11.8	<0.01
Group 1[3]	38.7	46.4	39.3	46.2	44.7	2.30	0.02
Group 2[4]	139^{b}	174^{ab}	219^{a}	184^{ab}	176^{ab}	10.9	<0.02
BC-Group 2[5]	78^{c}	100^{bc}	161^{a}	124^{b}	89^{bc}	8.0	<0.01
NB-Group 2[6]	61.3^{b}	73.9^{ab}	58.3^{b}	59.6^{b}	86.9^{a}	4.20	<0.01
NEAA[7]	145	124	116	119	136	13.5	0.53
TAA[8]	313	333	365	339	346	22.6	0.57
Arg	18.9	21.5	20.1	19.7	23.5	1.08	0.10
His	7.2	8.5	7.4	8.4	8.6	0.57	0.13
Ile	20.4^{c}	27.2^{bc}	39.9^{a}	33.6^{ab}	23.6^{c}	2.03	<0.01
Leu	30.1^{d}	40.9^{bc}	60.6^{a}	48.8^{b}	35.0^{cd}	2.49	<0.01
Lys	26.2^{bc}	34.1^{ab}	22.3^{c}	23.2^{c}	42.8^{a}	2.81	<0.01
Met	6.9	8.3	6.8	7.9	7.8	0.45	0.03
Phe	12.0^{b}	14.9^{a}	11.9^{b}	15.8^{a}	14.2^{ab}	0.74	<0.01
Thr	16.2^{b}	18.4^{ab}	15.8^{b}	16.7^{ab}	20.5^{a}	1.06	0.02
Trp	2.5	2.9	3.0	3.2	2.9	0.25	0.54
Val	27.0^{b}	32.1^{b}	60.4^{a}	42.0^{b}	30.2^{b}	3.78	<0.01
Ala	25.1^{a}	6.4^{ab}	12.8^{ab}	4.8^{b}	7.5^{ab}	4.32	0.03
Asn	7.0^{ab}	8.7^{a}	5.3^{b}	7.7^{ab}	8.2^{a}	0.65	0.01
Asp	1.2	0.1	0.8	0.9	1.5	0.30	0.06
Cit	1.3^{ab}	0.2^{b}	2.8^{a}	1.7^{ab}	1.2^{ab}	0.55	0.07
Cys	0.12^{b}	0.34^{ab}	0.18^{ab}	0.54^{a}	0.25^{ab}	0.083	0.03
Gln	40.5	41.4	30.8	44.4	44.8	5.22	0.36
Glu	21.4	16.6	20.1	15.7	21.9	2.64	0.23
Gly	13.1	9.3	11.6	8.1	9.8	3.07	0.78
Orn	10.1	10.0	10.4	9.1	14.5	1.50	0.14
Pro	8.0^{a}	5.6^{b}	6.2^{ab}	5.2^{b}	6.1^{ab}	0.51	0.02
Ser	7.3	13.5	5.0	10.4	6.9	3.15	0.16
Tyr	10.0	11.7	10.2	10.9	11.4	0.70	0.32

$^{a-d}$Means within a row with no common superscripts differ ($P < 0.05$).

[1]Abomasal infusion treatments were saline (negative control) and 4 different AA profiles. SAL = 0.9% saline infusion; EAAC = a complete mixture of EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); ILV = mixture of Ile, Leu, and Val; GR1+ILV = mixture of His, Ile, Leu, Met, Phe, Trp, and Val; GR1+ALT = mixture of Arg, His, Lys, Met, Phe, Thr, and Trp. Amino acids within treatments were infused in amounts relative to their content in casein. All AA treatments supplied 562 g of MP/d, and solutions were delivered in 15-L batches.

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

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

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

[5]BC-Group 2 = Ile, Leu, Val.

[6]NB-Group 2 = Arg, Lys, Thr.

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

[8]TAA = EAA + NEAA.

Mammary Gland AA U:O

Infusion of EAAC did not affect the mammary gland U:O of any EAA group or individual EAA compared with SAL ($P > 0.95$; Table 7). Infusion of ILV increased U:O of EAA, group 2, and BC-group 2 AA (as a group and individually) over SAL and all other AA infusions ($P < 0.01$). Individually, U:O of Ile and Leu increased and U:O of Thr decreased with GR1+ILV compared with SAL ($P \leq 0.05$), U:O of Arg decreased with GR1+ILV compared with ILV and GR1+ALT ($P \leq 0.02$), and U:O of Lys decreased with GR1+ILV compared with EAAC ($P = 0.03$). Infusion of GR1+ALT increased U:O of NB-group 2 AA and individual U:O of Lys over SAL and all AA infusions ($P \leq 0.05$), and increased U:O of Thr over EAAC and GR1+ILV ($P \leq 0.04$).

Infusion of EAAC decreased U:O of NEAA and individual U:O of Ala, Asp, and Pro compared with SAL ($P \leq 0.05$). Compared with SAL, infusion of GR1+ILV decreased U:O of NEAA and individual U:O of Ala and Pro ($P \leq 0.03$), and increased U:O of Cys ($P = 0.02$). Infusion of GR1+ALT decreased individual U:O of Ala and Pro compared with SAL ($P \leq 0.05$). Infusion of

Table 6. Mammary clearances (L/h) of AA groups in lactating dairy cows receiving 5-d abomasal infusions of saline (SAL) or 562 g/d of AA as a complete mixture of EAA (EAAC), as a mixture of Ile, Leu, and Val (ILV), as a mixture of group 1 AA and Ile, Leu, and Val (GR1+ILV), and as a mixture of group 1 AA and Arg, Lys, and Thr (GR1+ALT)

	Treatment[1]						
Item	SAL	EAAC	ILV	GR1+ILV	GR1+ALT	SEM	*P*-value
EAA[2]	400[a]	215[b]	145[b]	190[b]	226[b]	30.1	<0.01
Group 1[3]	409[ab]	237[bc]	575[a]	127[c]	142[c]	54.0	<0.01
Group 2[4]	421[a]	225[b]	134[b]	222[b]	287[ab]	40.5	<0.01
BC-Group 2[5]	355[ab]	190[bc]	105[c]	166[bc]	475[a]	46.3	<0.01
NB-Group 2[6]	566[bc]	312[cd]	689[ab]	863[a]	249[d]	65.8	<0.01
NEAA[7]	155	163	124	125	134	16.5	0.32
TAA[8]	227[a]	190[ab]	136[b]	157[ab]	180[ab]	16.8	0.02

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

[1]Abomasal infusion treatments were saline (negative control) and 4 different AA profiles. SAL = 0.9% saline infusion; EAAC = a complete mixture of EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); ILV = mixture of Ile, Leu, and Val; GR1+ILV = mixture of His, Ile, Leu, Met, Phe, Trp, and Val; GR1+ALT = mixture of Arg, His, Lys, Met, Phe, Thr, and Trp. Amino acids within treatments were infused in amounts relative to their content in casein. All AA treatments supplied 562 g of MP/d, and solutions were delivered in 15-L batches.

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

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

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

[5]BC-Group 2 = Ile, Leu, Val.

[6]NB-Group 2 = Arg, Lys, Thr.

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

[8]TAA = EAA + NEAA.

ILV increased U:O of TAA over EAAC and GR1+ILV ($P \leq 0.02$) and increased TAA U:O expressed on an N basis over all other AA infusions ($P \leq 0.03$). Total AA U:O expressed on an N basis was greater than 1 during ILV infusion, tended to differ from 1 during EAAC infusion, but did not differ from 1 on all other treatments ($P \leq 0.10$).

Glucose Metabolism and $^{13}CO_2$ Production

Arterial plasma glucose concentration, arterial glucose IE, and mammary net uptake of glucose were not affected by AA infusions ($P \geq 0.11$; Table 8). Infusion of GR1+ALT increased glucose WB Ra over SAL and ILV ($P = 0.05$). The calculated glucose required for lactose and fat synthesis was not affected by treatment ($P \geq 0.23$), and mammary glucose uptake was sufficient to cover these estimated requirements (i.e., glucose balance across treatments did not differ from zero; $P \geq 0.10$). Neither lactose output as a proportion of mammary glucose uptake nor as a proportion of WB Ra was affected by treatment ($P \geq 0.39$), averaging 0.67 and 0.53, respectively. The proportion of mammary glucose uptake relative to WB Ra was not affected by treatment ($P = 0.89$) and averaged 0.76. The IE of glucose and galactose in milk lactose and the ratio of galactose IE relative to glucose IE (average 0.72) was not affected by treatment ($P \geq 0.74$). Isotopic enrichment of glucose in milk lactose was higher than IE of galactose ($P < 0.01$). Total $^{13}CO_2$ production (Figure 1) between 0500 h on d 5 and 0500 h on d 6 did not differ between treatments ($P = 0.57$), and averaged 31.4, 28.8, 32.5, 33.6, and 27.0 mmol for SAL, EAAC, ILV, GR1+ILV, and GR1+ALT, respectively, with an average SEM of 3.61. Whole-body glucose oxidation and the proportion of WB glucose oxidation relative to WB Ra were not affected by treatment ($P \geq 0.45$) and averaged 118 mmol/h and 0.17, respectively, across treatments.

DISCUSSION

The current results complement previously reported energy and N balance from the same study (Nichols et al., 2019a). Examinations of the effects of incomplete EAA profiles on mammary gland metabolism have been conducted under conditions where single AA or groups of AA are subtracted from 5 to 14 d postruminal infusions of complete AA profiles (Bequette et al., 2000; Doepel and Lapierre, 2011; Doepel et al., 2016). However, the reduced MP supply from the subtracted AA in these studies is not compensated. Therefore, the first objective of the present work was to examine mammary gland responses to incomplete EAA profiles at a constant supplemental MP level for 5 d, with the

Table 7. Mammary gland AA uptake to milk output ratios in lactating dairy cows receiving 5-d abomasal infusions of saline (SAL) or 562 g/d of AA as a complete mixture of EAA (EAAC), as a mixture of Ile, Leu, and Val (ILV), as a mixture of group 1 AA and Ile, Leu, and Val (GR1+ILV), and as a mixture of group 1 AA and Arg, Lys, and Thr (GR1+ALT)

	Treatment[1]						
Item	SAL	EAAC	ILV	GR1+ILV	GR1+ALT	SEM	*P*-value
EAA[2]	1.23^{b}	1.28^{b}	1.84^{a}	1.35^{b}	1.35^{b}	0.078	<0.01
Group 1[3]	1.01*	1.00*	1.01*	1.00*	1.01*	0.013	0.83
Group 2[4]	1.28^{b}	1.34^{b}	2.04^{a}	1.42^{b}	1.43^{b}	0.097	<0.01
BC-Group 2[5]	1.20^{b}*	1.29^{b}	2.52^{a}	1.61^{b}	1.23^{b}*	0.138	<0.01
NB-Group 2[6]	1.40^{b}	1.41^{b}	1.32^{b}	1.13^{b}†	1.72^{a}	0.066	<0.01
NEAA[7]	0.80^{a}	0.55^{b}	0.58^{ab}	0.53^{b}	0.62^{ab}	0.055	0.03
TAA[8]	0.98^{ab}*	0.86^{b}	1.11^{a}	0.88^{b}	0.91^{ab}*	0.044	0.01
TAA-N[9]	1.01^{ab}*	0.91^{b}†	1.20^{a}	0.93^{b}*	0.96^{b}*	0.045	<0.01
Arg	2.55^{ab}	2.40^{ab}	2.69^{a}	2.20^{b}	2.68^{a}	0.094	0.01
His	1.10	1.07*	1.10	1.07*	1.14	0.043	0.74
Ile	1.25^{c}*	1.40^{bc}	2.48^{a}	1.72^{b}	1.29^{bc}†	0.147	<0.01
Leu	1.09^{c}*	1.24^{bc}	2.24^{a}	1.48^{b}	1.12^{bc}*	0.112	<0.01
Lys	1.22^{bc}†	1.33^{ab}	1.04^{bc}*	0.90^{c}*	1.75^{a}	0.111	<0.01
Met	1.00*	1.00*	0.98*	0.96*	0.98*	0.029	0.67
Phe	1.10	1.13	1.08	1.19	1.15	0.034	0.16
Thr	1.09^{ab}	1.02^{bc}*	1.04^{abc}*	0.93^{c}†	1.19^{a}	0.033	<0.01
Trp	0.89*	0.84	1.00*	0.91*	0.84	0.064	0.42
Val	1.29^{b}*	1.29^{b}*	2.91^{a}	1.68^{b}	1.32^{b}*	0.182	<0.01
Ala	1.87^{a}	0.35^{b}	0.97^{ab}*	0.26^{b}	0.41^{b}†	0.303	0.01
Asn	0.59	0.62	0.44	0.55	0.61	0.043	0.06
Asp	0.07^{a}	0.01^{b}	0.05^{ab}	0.05^{ab}	0.08^{ab}	0.016	0.04
Cys	0.05^{b}	0.11^{ab}	0.06^{ab}	0.16^{a}	0.08^{ab}	0.023	0.03
Gln	1.70	1.42	1.25*	1.53	1.58	0.182	0.48
Glu	0.52	0.33	0.48	0.31	0.47	0.061	0.10
Gly	1.49*	0.82*	1.21*	0.71*	0.90*	0.299	0.37
Pro	0.25^{a}	0.14^{b}	0.19^{ab}	0.13^{b}	0.15^{b}	0.017	<0.01
Ser	0.36	0.51	0.19	0.40	0.26	0.126	0.23
Tyr	0.90	0.87	0.92	0.81	0.89	0.033	0.16

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

[1]Abomasal infusion treatments were saline (negative control) and 4 different AA profiles. SAL = 0.9% saline infusion; EAAC = a complete mixture of EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); ILV = mixture of Ile, Leu, and Val; GR1+ILV = mixture of His, Ile, Leu, Met, Phe, Trp, and Val; GR1+ALT = mixture of Arg, His, Lys, Met, Phe, Thr, and Trp. Amino acids within treatments were infused in amounts relative to their content in casein. All AA treatments supplied 562 g of MP/d, and solutions were delivered in 15-L batches.

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

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

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

[5]BC-Group 2 = Ile, Leu, Val.

[6]NB-Group 2 = Arg, Lys, Thr.

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

[8]TAA = EAA + NEAA.

[9]TAA on a N basis.

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

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

general hypothesis that intramammary metabolism of the supplemented EAA in the incomplete profiles would increase in support of milk protein synthesis.

Mammary Plasma Flow

Mammary plasma flow was fastest during ILV infusion, increasing 38% over that on EAAC and GR1+ILV. Mammary plasma flow can be altered during excessive or deficient supply of milk precursors in an attempt to maintain extracellular and intracellular concentrations of precursor substrates required for milk component synthesis, particularly when single EAA, acetate, or BHB are deficient in arterial circulation (Cant et al., 2003). In previous studies, extra MP from EAA and NEAA (Galindo et al., 2011; Nichols et al., 2019b), or

Table 8. Whole-body and mammary glucose metabolism in lactating dairy cows receiving 5-d abomasal infusions of saline (SAL) or 562 g/d of AA as a complete mixture of EAA (EAAC), as a mixture of Ile, Leu, and Val (ILV), as a mixture of group 1 AA and Ile, Leu, and Val (GR1+ILV), and as a mixture of group 1 AA and Arg, Lys, and Thr (GR1+ALT)

	Treatment[1]						
Item	SAL	EAAC	ILV	GR1+ILV	GR1+ALT	SEM	*P*-value
Arterial glucose, m*M*	3.64	3.64	3.58	3.36	3.71	0.140	0.49
Arterial glucose IE, APE[2]	0.528	0.507	0.525	0.472	0.459	0.0261	0.11
WB Ra,[3] mmol/h	651[b]	700[ab]	658[b]	728[ab]	757[a]	33.9	0.04
Mammary glucose uptake, mmol/h	553	549	528	510	605	81.0	0.94
Glucose required for lactose,[4] mmol/h	359	392	372	397	391	23.5	0.25
Glucose required for fat,[4] mmol/h	101	99	107	115	104	7.0	0.23
Mammary glucose balance,[5] mmol/h	94	59	48	−2	94	70.8	0.86
Lactose output[6]:mammary glucose uptake	0.64	0.73	0.60	0.74	0.62	0.084	0.39
Mammary glucose uptake:WB Ra	0.82	0.75	0.78	0.70	0.76	0.122	0.89
Lactose output[7]:WB Ra	0.51	0.57	0.54	0.52	0.51	0.032	0.73
WB glucose oxidation, mmol/h	124	113	113	105	134	11.7	0.55
WB glucose oxidation:WB Ra	0.19	0.16	0.18	0.15	0.18	0.018	0.45
Lactose IE							
Glucose,[8] APE	0.149	0.134	0.134	0.126	0.121	0.0167	0.74
Galactose, APE	0.108	0.100	0.096	0.089	0.088	0.0135	0.81
Galactose IE/glucose IE	0.71	0.73	0.71	0.70	0.73	0.025	0.83

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

[1]Abomasal infusion treatments were saline (negative control) and 4 different AA profiles. SAL = 0.9% saline infusion; EAAC = a complete mixture of EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); ILV = mixture of Ile, Leu, and Val; GR1+ILV = mixture of His, Ile, Leu, Met, Phe, Trp, and Val; GR1+ALT = mixture of Arg, His, Lys, Met, Phe, Thr, and Trp. Amino acids within treatments were infused in amounts relative to their content in casein. All AA treatments supplied 562 g of MP/d, and solutions were delivered in 15-L batches.

[2]IE = isotopic enrichment; APE = atom percent excess.

[3]WB = whole body; Ra = rate of glucose appearance.

[4]Based on milk lactose and fat yield from the afternoon milking on d 4. Requirements for lactose and fat estimated based on calculations of Dijkstra et al. (1996).

[5]Glucose balance = glucose uptake – glucose required for lactose – glucose required for fat. Values do not differ from zero ($P > 0.10$).

[6]Based on milk lactose yield from the afternoon milking on d 4.

[7]Based on milk lactose yield from the afternoon milking on d 5.

[8]Difference between glucose APE and galactose APE differs from zero ($P < 0.01$).

as a mixture of EAA only (Nichols et al., 2019c), did not affect MPF compared with no extra MP, which is in line with the result of the present experiment. However, the effects of EAA deletions on MPF are variable. In dairy cattle, removal of Lys from an abomasally infused complete AA profile infused for 7 d did not affect MPF (Lapierre et al., 2009), whereas removal of Thr from a complete AA profile infused for 10 d increased MPF by 50% (Doepel et al., 2016). Deletion of Arg from a complete EAA profile infused for 14 d did not affect MPF compared with the complete profile, but decreased it 17% relative to control levels (Doepel and Lapierre, 2011). Haque et al. (2015) postruminally infused an EAA profile missing Thr and Trp and observed no effect on MPF in dairy cattle after 14 d, whereas Bequette et al. (2000) reported a 33% increase in MPF in goats when His was removed from a 7-d abomasal infusion of AA in the profile of microbial protein. In the current study, if MPF was responding to the absence of any single EAA, it should have also increased during the other incomplete infusions (i.e., GR1+ILV and GR1+ALT). The magnitude of effect on MPF when single EAA are removed from complete supplements could relate to the degree of change in arterial concentration of the other EAA (Cant et al., 2001). The ILV infusion was relatively more imbalanced than GR1+ILV and GR1+ALT with respect to a complete EAA profile, and this may explain why MPF was only increased in response to ILV. This difference in magnitude of imbalance can be seen in the response of circulating EAA concentrations to the incomplete infusions, where ILV increased the circulating concentration of only the BC-group 2 AA compared with SAL, and GR1+ILV and GR1+ALT increased the concentration of all EAA (numerical increase only for Ile with GR1+ILV infusion) in the infusate profile compared with SAL. Changes in blood flow are often associated with concomitant changes in arterial concentration of acetate and BHB, particularly during glucogenic infusions where the arterial concentrations of these metabolites decrease and MPF increases (Raggio et al., 2006; Curtis et al., 2018; Nichols et al., 2019c). A link between AA supply and MPF has been hypothesized to lie with the associated effects on arterial energy me-

tabolites when EAA supply is increased (Cant et al., 2003, 2018). However, arterial BHB concentration was not affected by the infused AA profiles in the current study (Supplemental Table S2). This adds credence to the suggestion that the effect of ILV infusion on MPF is related to the relative AA imbalance, and may indicate that the mechanisms regulating MPF under conditions of AA imbalance can override those responsible for maintaining the ATP status of the gland.

Intramammary Flexibility During ILV Infusion

Arterial concentration of BC-group 2 AA during ILV infusion was increased over EAAC and GR1+ILV (treatments where Ile, Leu, and Val were also infused), but AVD of this AA group during ILV infusion did not differ from that on EAAC and GR1+ILV. The higher uptake of BC-group 2 AA can hence be attributed to the faster MPF during ILV infusion. The U:O of BC-group 2 AA increased with ILV over all infusions, to approximately double the level observed on SAL, EAAC, and GR1+ALT, and 1.6-times that on GR1+ILV, in agreement with our hypothesis that intramammary catabolism of BC-group 2 AA would be elevated when they were infused in the absence of other EAA. Consistent with previous research [e.g., Bequette et al. (1996) who reported oxidation of 11–23% of mammary Leu uptake], this observation shows that a high level of intramammary catabolism of Ile, Leu, and Val is possible by the lactating mammary gland, at least over a 5-d period. The mammary gland obtains substantial N and carbon for de novo NEAA synthesis from catabolism of BC-group 2 AA taken up in excess relative to their output in milk (Lapierre et al., 2012). The U:O of total AA-N increased with ILV over the other AA infusions, driven by the increased U:O of BC-group 2 AA, and unlike all other treatments was significantly greater than 1. The substantial difference in mammary uptake of BC-group 2 AA and consequently total AA-N, relative to their output in milk, suggests that catabolic products of Ile, Leu, and Val likely contributed to other mammary pathways (lactose and fat synthesis, and oxidation) above what was used for intramammary NEAA synthesis. This increased intramammary AA catabolism supports the lowest milk N efficiency observed on this treatment (Nichols et al., 2019a) and the observation that despite the lower DMI, milk protein, fat, and lactose production did not differ between ILV and SAL. Further, sequestration of BC-group 2 AA in the mammary gland through accumulation in constitutive protein, their release as peptides, or their output in milk as free AA or peptides would all contribute to the U:O of total AA-N of >1, and would indicate that the mammary gland can function as an important clearance organ for BC-group 2 AA.

Intramammary Group 2 AA Flexibility During GR1+ILV and GR1+ALT Infusion

In line with stoichiometric transfer of their uptake into milk protein, mammary net uptake of group 1 AA did not differ between EAAC, GR1+ILV, and GR1+ALT. Differences in uptake of the group 2 AA were found between the incomplete infusions, where BC-group 2 AA uptake tended to be higher on GR1+ILV compared with GR1+ALT, and NB-group 2 AA uptake was higher on GR1+ALT compared with GR1+ILV. Infusion of GR1+ILV increased clearance of NB-group 2 AA (as a group and individually; Supplemental Table S1) over EAAC. Infusion of GR1+ALT increased mammary clearance of BC-group 2 AA and individual clearances of Leu and Val (Supplemental Table S1) over all other AA infusions. Because their uptakes were not different from EAAC, higher clearance of these EAA groups is due to their relatively lower arterial concentrations on the respective incomplete EAA infusions, and reflects mammary affinity for their sequestration over 5 d when supplementation of the other EAA stimulated milk protein synthesis.

We hypothesized that the same level of milk protein synthesis between EAAC, GR1+ILV (in the absence of Arg, Lys, and Thr), and GR1+ALT (in the absence of Ile, Leu, and Val) was maintained through intramammary compensation between the group 2 AA over the 5-d infusion period. Intramammary catabolism of NB-group 2 AA tended to decrease (and tended toward unity) with GR1+ILV compared with EAAC. The U:O of BC-group 2 AA numerically increased 25% over EAAC with GR1+ILV infusion. Therefore, intramammary catabolism of BC-group 2 AA could have compensated for lower levels of NB-group 2 AA during GR1+ILV infusion, in agreement with our hypothesis. During GR1+ALT infusion, intramammary catabolism of NB-group 2 AA increased over EAAC, but the U:O of BC-group 2 AA (individually and as a group) was not different from EAAC during GR1+ALT infusion. This difference in intramammary catabolism of the EAA groups absent from the respective infusions (i.e., NB-group 2 AA catabolism tended to decrease on GR1+ILV compared with EAAC, whereas BC-group 2 AA catabolism was not different from EAAC on GR1+ALT) arose primarily from the decrease in intramammary catabolism of Lys during GR1+ILV infusion. The U:O of Lys on GR1+ILV dropped from 1.33 on EAAC to 0.90 (not different from unity). When considered together with the U:O of Lys that was ob-

served on ILV (1.04; not different from unity), our findings suggest that when intramammary BC-group 2 AA levels are high, Lys catabolism by the gland becomes minor or not obligate. Similarly, Lapierre et al. (2009) removed Lys from a complete AA mixture and observed reduced U:O of Lys accompanied by reduced Lys-N transfer into NEAA, whereas milk protein yield was not affected. In particular, abundant intramammary levels of BC-group 2 AA or Arg could have compensated for the reduced contribution of Lys to intramammary amino-N exchanges in support of milk protein synthesis during GR1+ILV and ILV infusion (Bequette et al., 2003). Intracellular requirements for Arg appear to hold a constant level of priority for intramammary catabolism across a range of digestive supplies (33 to 175 g/d; Lapierre et al., 2012). Indeed, despite being lower on GR1+ILV compared with GR1+ALT, U:O of Arg was maintained >2 across all treatments, regardless of AA profile. Additionally, products of Lys catabolism formed in other tissues could have circulated to the mammary gland (Bequette et al., 2003; Lapierre et al., 2009). These, along with plasma peptides or breakdown products of mammary constituent protein, would not have been captured in our arteriovenous measurements but may have contributed to intramammary requirements for N and C skeletons (Bequette et al., 1996).

Mammary Lys Uptake may be Inhibited by High Concentrations of Ile and Leu

Because milk protein yield did not differ between EAAC and GR1+ILV, lower mammary U:O of Lys with GR1+ILV infusion compared with EAAC arose from the lower mammary uptake of Lys. Arterial concentrations of Arg, Lys, and Thr were lower on GR1+ILV compared with EAAC, but only Lys uptake was affected. This suggests that Lys uptake may have been inhibited through a mechanism that did not affect other NB-group 2 AA. Arterial Lys concentration was not different between SAL, ILV, and GR1+ILV. Considering the similarly lower uptake of Lys observed on ILV infusion compared with EAAC, it is compelling to suggest that the presence of high levels of arterial BC-group 2 AA (i.e., 1,021 and 1,933 μM on GR1+ILV and ILV, respectively, compared with 753 μM on EAAC) inhibited Lys transport over the 5-d infusion period. Yoder et al. (2019) suggested that extracellular concentrations of AA in varying profiles affected Lys uptake into mammary cells measured in vitro. Cationic Na^+-independent AA transport systems facilitate transfer of both Arg and Lys into bovine mammary cells (Baumrucker, 1985), and react with neutral AA, including Leu (Shennan et al., 1997). Lysine may also be transported into mammary cells using Na^+-dependent systems $ATB^{0,+}$ and y^+LAT1, both identified in porcine mammary tissue (Laspiur et al., 2009; Manjarin et al., 2011), and Na^+-independent LAT1 systems that have been identified in bovine mammary tissue (Bionaz and Loor, 2011), all of which are shared by Ile and Leu. When their arterial supply to mammary cells was high during ILV and GR1+ILV, it is possible that high intra- and extracellular concentrations of Ile and Leu reduced the transport of Lys into mammary cells via their shared transporters. The fact that Lys uptake may be more susceptible than Arg to competition for cellular transport further supports its apparent nonobligate intramammary metabolism compared with Arg (Lapierre et al., 2012).

Glucose Metabolism

Whole-body glucose flux typically increases in response to increased absorptive supply of AA, but this has predominantly been investigated in response to complete AA profiles (Clark et al., 1977; Lemosquet et al., 2009b; Galindo et al., 2011). We hypothesized that differences in affinity of certain AA groups for hepatic metabolism may affect glucose appearance during 5-d infusion of incomplete EAA profiles. Therefore, a secondary objective of this experiment was to determine if the profile of supplemented EAA affects WB Ra of glucose. Whole-body glucose Ra increased 8, 12, and 16% over SAL with EAAC, GR1+ILV, and GR1+ALT, respectively (numerical increases only for EAAC and GR1+ILV), and did not differ appreciably between ILV and SAL. Hepatic gluconeogenesis drives the increment in glucose appearance with increased MP supply (Galindo et al., 2011). Liver net uptake of group 1 AA, particularly Met, His, and Phe, is stimulated by increased protein intake (Raggio et al., 2004; Cantalapiedra-Hijar et al., 2014; Omphalius et al., 2020). The relative increases in WB Ra of glucose over SAL with EAAC, GR1+ILV, and GR1+ALT reflect the inclusion level of group 1 AA in those treatments, where His, Met, and Phe could have contributed to glucose appearance via hepatic gluconeogenesis. Body protein turnover and hepatic AA catabolism stimulated by glucagon in response to EAA infusions (Danfær et al., 1995) also could have provided endogenous sources of AA-C for gluconeogenesis, particularly during ILV infusion where DMI decreased 1.3 kg/d compared with SAL (Nichols et al., 2019a) and the arterial concentration of Ala decreased relative to SAL. Liver extraction of glucogenic branched-chain AA Ile and Val is negligible across a range of MP supplies (Raggio et al., 2004; Omphalius et al., 2020). As such, the relative contribution of infused EAA to gluconeogenesis during ILV infusion was expected to have been less compared with

the treatments supplying other EAA, as Ile and Val would first be exposed to utilization by extrahepatic tissues before their catabolic products would be used for gluconeogenesis in the liver (Brosnan and Brosnan, 2006). Catabolism of endogenous AA in support of glucose synthesis during ILV infusion agrees with the lowest N efficiency and plasma insulin concentration observed on this treatment (Nichols et al., 2019a), as reduced plasma insulin favors proteolysis and gluconeogenesis.

The majority of WB glucose flux in a lactating dairy cow is used by the mammary glands for milk synthesis (Bickerstaffe et al., 1974). Mammary glucose uptake accounted for on average 76% of WB Ra of glucose across all treatments, which is consistent with Galindo et al. (2011) who observed that 73% of WB Ra of glucose was used by the mammary gland during infusions of AA or casein. Lactose synthesis accounts for the majority of mammary-sequestered glucose, and oxidation of the remaining glucose facilitates energy and precursor supply for synthesis of TAG and proteins (Bickerstaffe et al., 1974; Xiao and Cant, 2005). Mammary glucose uptake sufficiently covered theoretical glucose requirements for lactose and fat synthesis across all treatments in the current study, and mammary glucose balances did not differ from zero. In nonmammary tissues, postabsorptive glucose will be used predominantly for oxidation and TAG synthesis (Danfær et al., 1995). Whole-body glucose oxidation accounted for on average 17% of WB Ra of glucose, which agrees with the proportion of WB glucose flux directed to oxidation observed by Bauman et al. (1988; 17.4%) in dairy cattle consuming a 15.9% CP diet, and slightly higher than that observed by Clark et al. (1977) during abomasal infusion of water (13.4%) or sodium caseinate (7.8%). Underlying our WB glucose oxidation estimate is the assumption that 30% of ^{13}C was retained (Junghans et al., 2007) and did not appear in the $^{13}CO_2$ measurements over the 24-h time period analyzed. Bicarbonate formation will vary with energy metabolism across physiological conditions; thus, WB glucose oxidation may be over- or underestimated depending on the dynamics of bicarbonate metabolism under the conditions of this experiment. Interestingly, negligible oxidation of mammary glucose uptake (i.e., glucose balance being numerically very close to zero) was observed with GR1+ILV infusion, and this treatment also produced numerically the lowest WB glucose oxidation as a proportion of WB Ra (15%). Because the mammary gland is the greatest net user of glucose in the body of a dairy cow, particularly in early to mid-lactation, it follows that intramammary glucose oxidation would influence total CO_2 produced from glucose at the WB level.

Bovine mammary cells are unable to convert gluconeogenic substrates to glucose due to the virtual absence of glucose-6-phosphatase in mammary tissue (Scott et al., 1976). Thus, the glucose moiety of milk lactose primarily arises from free glucose taken up by the gland, but synthesis of the galactose moiety is possible in part from nonglucose hexose phosphate intermediates via the pentose phosphate pathway (Wood et al., 1965). However, the contribution of nonglucose precursors to milk galactose synthesis is apparently not fixed, with ratios of galactose IE to glucose IE of 0.52 and 0.83 reported by Maxin et al. (2013) and Lapierre et al. (2013), respectively, in response to postruminal EAA infusions. Using bovine mammary explants incubated with [U-^{13}C] glucose, Bequette et al. (2006) reported that up to 86% of galactose in lactose was synthesized de novo from nonglucose carbon sources, and estimated that as much as 12% of galactose was derived from EAA catabolism. The observed ^{13}C enrichment of lactose fractions in the current study suggests that approximately 28% of milk galactose arose from nonglucose precursors, with or without EAA supplementation and regardless of EAA profile. It appears that even when mammary glucose supply does not limit milk lactose or fat synthesis, AA carbon contributes to galactose synthesis, and that this contribution remains relatively constant when the EAA profile of supplemental MP is varied.

CONCLUSIONS

When Arg, Lys, and Thr, or Ile, Leu, and Val were absent from 5-d postruminal EAA infusions where the other 7 EAA equalized the MP supply, intramammary catabolism of the present group 2 AA compensated for lower mammary uptake of the absent EAA. During infusion of ILV and GR1+ILV, intramammary catabolism of branched-chain AA and Arg likely compensated for the contribution of Lys to oxidation and NEAA synthesis, suggesting that Lys catabolism is nonobligate for milk protein synthesis in mammary glands of dairy cattle. Overall, these findings illustrate flexibility in mammary net uptake and intramammary utilization of group 2 AA for oxidation and amino-N exchanges to support milk component synthesis when the EAA profile of MP is incomplete with respect to casein. Further, compared with a saline control, the increase in WB Ra of glucose with GR1+ALT and the numerical increases with EAAC and GR1+ILV reflect the inclusion level of group 1 AA in those infusions. Across all treatments, mammary gland glucose uptake accounted for on average 76% of WB Ra of glucose, and sufficiently covered mammary glucose requirements for milk component synthesis. Finally, on average 28% of milk galactose

arose from nonglucose precursors, regardless of EAA profile infused.

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ORCIDS

K. Nichols https://orcid.org/0000-0001-6062-7460
J. Dijkstra https://orcid.org/0000-0003-3728-6885
S. Lemosquet https://orcid.org/0000-0002-1077-1285
W. J. J. Gerrits https://orcid.org/0000-0003-0494-9259
A. Bannink https://orcid.org/0000-0001-9916-3202