Mammary gland metabolite utilization in response to exogenous glucose or long-chain fatty acids at low and high metabolizable protein levels

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ABSTRACT

We investigated mammary gland metabolism in lactating dairy cattle in response to energy from glucogenic (glucose; GG) or lipogenic (palm olein; LG) substrates at low (LMP) and high (HMP) metabolizable protein levels. According to a 6 × 6 Latin square design, 6 rumen-fistulated second-lactation Holstein-Friesian dairy cows (97 ± 13 d in milk) were abomasally infused with saline (LMP-C); isoenergetic infusions (digestible energy basis) of 1,319 g/d glucose (LMP-GG), 676 g/d palm olein (LMP-LG), or 844 g/d essential AA (EAA; HMP-C); or isoenergetic infusions of 1,319 g/d glucose + 844 g/d EAA (HMP-GG) or 676 g/d palm olein + 844 g/d EAA (HMP-LG). Each experimental period consisted of 5 d of continuous infusion followed by 2 d of rest. A total mixed ration (42% corn silage, 31% grass silage, and 27% concentrate on a dry matter basis) formulated to meet 100 and 83% of net energy and metabolizable protein requirements, respectively, was fed at 90% of ad libitum intake by individual cow. Arterial and venous blood samples were collected on d 5 of each period. Infusing GG or LG at the HMP level did not affect milk yield or composition differently than at the LMP level. Neither GG nor LG infusion stimulated milk protein or lactose yield, but fat yield tended to decrease with GG and tended to increase with LG. Infusion of GG increased arterial plasma concentrations of glucose and insulin and decreased concentrations of β-hydroxybutyrate (BHB), nonesterified fatty acids, long-chain fatty acids (LCFA), total AA, EAA, and group 2 AA. Infusion of LG increased arterial triacylglycerides (TAG) and LCFA but did not affect EAA concentrations. Compared with the LMP level, the HMP level increased arterial concentrations of BHB, urea, and all EAA groups and decreased the concentration of total non-EAA. Mammary plasma

flow increased with GG and was not affected by LG or protein level. Uptake and clearance of total EAA and group 2 AA were affected or tended to be affected by GG × AA interactions, with their uptakes being lower and their clearances higher with GG, but only at the LMP level. Infusion of LG did not affect uptake or clearance of any AA group. The HMP level increased uptake and decreased clearance of all EAA groups and decreased non-EAA uptake. Infusion of GG tended to increase mammary glucose uptake, and tended to decrease BHB uptake only at the LMP level. Infusion of LG increased mammary uptake of TAG and LCFA and increased or tended to increase clearance of TAG and LCFA. We suspect GG increased mammary plasma flow to maintain intramammary energy and AA balance and stimulated lipogenesis in adipose, accounting for depressed arterial BHB and group 2 AA concentrations. Mammary glucose uptake did not cover estimated requirements for lactose and fat synthesis at the HMP level, except during HMP-GG infusion. Results of this study illustrate flexibility in mammary metabolite utilization when absorptive supply of glucogenic, lipogenic, and aminogenic substrate is increased. **Key words:** glucogenic, lipogenic, mammary gland, essential amino acid, milk synthesis

INTRODUCTION

Characterizing the effect of macronutrient supply on mammary gland metabolism of lactating dairy cows is important to achieve efficient and sustainable nutrient transfer from feed into milk components. Lactating mammary glands regulate local blood flow and transmembrane transport to extract substrates for milk component synthesis and to balance cellular ATP requirements in the presence of a deficiency or excess of energy metabolites (Cant et al., 2003). Glucogenic substrates in dairy diets are important to lactating ruminant metabolism because mammary glucose use is high, but net glucose absorption from the gastrointestinal tract is low (Huntington et al., 2006). Hyperinsulinemia caused by

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elevated circulating glucose concentrations or insulin infusion stimulates milk protein synthesis (Mackle et al., 2000; Bequette et al., 2001; Rulquin et al., 2004); however, this endocrine response may also be responsible for reduced arterial concentrations of EAA, acetate, BHB, and nonesterified fatty acids (**NEFA**) in mid- to late-lactation cows when extramammary peripheral tissues are insulin sensitive (Mackle et al., 1999; Curtis et al., 2018). On the other hand, lipogenic substrates improve the transfer of dietary energy into milk energy (van Knegsel et al., 2007; Boerman et al., 2015), but their metabolism does not provide a net supply of glucose. Lipogenic diets created through provision of high-fiber forages, supplementation of rumen-inert long-chain fatty acids (**LCFA**), or both may stimulate total milk, protein, and lactose production to the same or to a greater degree relative to glucogenic diets in early- and mid-lactation cows (van Knegsel et al., 2007; Hammon et al., 2008; Boerman et al., 2015), but lipogenic diets do not initiate the same insulin-stimulated endocrine cascade as glucogenic or aminogenic diets (van Hoeij et al., 2017; Nichols et al., 2019a).

Postruminal fat supplementation has received less attention relative to that of glucose with regard to its effect on mammary metabolite utilization. Early work described mammary metabolite use in response to largely unsaturated fat sources (Casper and Schingoethe, 1989; Cant et al., 1993a,b). Cant et al. (1993a) replaced a portion of cereal grains in the diet with a mix of saturated and unsaturated fats and observed increased mammary uptake of triacylglycerides (**TAG**) and output of LCFA in milk, higher lactose yield, and increased mammary glucose utilization for lactose. In the same study, AA arteriovenous (**AV**) differences increased but mammary blood flow decreased on high-fat diets (Cant et al., 1993b). Arieli et al. (2001) observed no difference in mammary extraction efficiency of AA with abomasal infusion of corn oil. Recently, Nichols et al. (2019a) reported that supplementing rumen-inert saturated fat did not affect mammary AA metabolism.

In the same experiment as described in the current work (Nichols et al., 2019b), we found that at low and high MP levels, arterial insulin concentration in midlactation cows was increased by abomasal infusion of glucose but was not affected by infusion of palm olein (major fatty acid [**FA**] constituents are palmitic, oleic, and linoleic acid). Furthermore, glucose infusion increased energy retention, reduced milk energy output, and improved milk N efficiency; palm olein infusion increased milk energy output and had no effect on energy retention and milk N efficiency; and EAA infusion increased milk energy output without affecting energy retention and decreased milk N efficiency. In the current study we measured mammary gland metabolite

utilization when glucose or palm olein were supplied at low and high MP levels. We hypothesized that glucose infusion would stimulate mammary gland mechanisms to sequester AA to a greater extent than palm olein. We expected the high MP level, achieved through EAA infusion, to increase mammary gland uptake of EAA as well as increase intramammary AA catabolism.

MATERIALS AND METHODS

Experimental Design

All experimental procedures were conducted under the Dutch Law on Animal Experiments in accordance with EU Directive 2010/63. The experimental design, animal housing, ration composition and preparation, and feed chemical analyses have been described in detail by Nichols et al. (2019b). Briefly, effects of energy from glucogenic (**GG**) or lipogenic (**LG**) substrates at low (**LMP**) and high (**HMP**) MP levels were tested according to a 3×2 factorial arrangement (absence or presence of supplemented energy from GG or LG $\times$ MP level) using 6 rumen-fistulated second-lactation Holstein-Friesian dairy cows (97 ± 13 DIM) randomly assigned to a 6×6 Latin square design, in which each experimental period consisted of 5 d of continuous abomasal infusion followed by 2 d of no infusion. 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 before the first experimental period began. Cows were fed a TMR (14% CP) consisting of 42% corn silage, 31% grass silage, and 27% concentrate on a DM basis that was formulated to meet 100 and 83% of NE_L and MP requirements (Centraal Veevoederbureau, 2008), respectively, for cows consuming 20 kg DM/d and producing 30 kg/d of milk containing 40 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 a 10-d diet adaptation period in tie stalls before cows entered the CRC. Fresh feed was allocated twice daily during the entire experiment, with the exception of a 34-h window over d 4 and 5 of each period (from 0530 h on d 4 until 1530 h on d 5), during which an automated feeding system dispensed equal portions of feed every 2 h to promote metabolic steady-state conditions in preparation for the blood sampling protocol described below.

Infusion lines were placed in the abomasum via the rumen cannula 7 d before the first experimental period and were checked daily for patency and position. Abomasal infusion treatments were (1) 0.9% saline (LMP-C; 90% NE_L , 75% MP), (2) 1,319 g/d glucose (LMP-GG; 100% NE_L , 75% MP), (3) 676 g/d palm

olein (LMP-LG; 100% NE_L, 75% MP), (4) 844 g/d EAA (HMP-C; 100% NE_L, 120% MP), (5) 1,319 g/d glucose + 844 g/d EAA (HMP-GG; 110% NE_L, 120% MP), and (6) 676 g/d palm olein + 844 g/d EAA (HMP-LG; 110% NE_L, 120% MP), in which MP and NE_L reflect the proportion of requirements met by the restricted feeding level of the diet plus the infusate. Treatment solutions were administered in 10-L batches that were replenished daily and infused continuously via multi-channel peristaltic pumps at a rate of 6.95 mL/min to facilitate 120-h of continuous infusion. Daily infusion dosages (g/d) of LMP-GG, LMP-LG, and HMP-C were designed to be isoenergetic based on the gross energy content of the EAA infusion, which was calculated to be 24.3 MJ/kg based on the heat of combustion of individual EAA in the infusate and assuming digestibility of palm olein, EAA, and glucose to be 80%, 100%, and 100%, respectively. The EAA infusions delivered EAA in the same profile and amount as found in 1.5 kg of casein according to Metcalf et al. (1996). Composition of the EAA mixture and palm olein FA composition are described by Nichols et al. (2019b).

Milk and Blood Collection and Analysis

Cows were milked twice daily at 0530 and 1530 h. Milk was collected, weighed, and sampled separately 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; VVB, Doetinchem, the Netherlands). At 0730, 0930, 1130, 1330, and 1530 h on d 5 of infusion, blood samples were collected into 10-mL sodium heparin and potassium EDTA Vacutainers (Becton Dickinson, Rutherford, NJ) from the coccygeal vessels and from the subcutaneous abdominal vein of each cow. Arteriovenous differences across the tail are assumed to be negligible and thus samples from the coccygeal vessels are representative of mammary arterial supply (Emery et al., 1965). Samples were collected from the left and right subcutaneous abdominal veins, alternating at each time point, to account for differences between sides. Collection tubes were immediately placed in ice and centrifuged at $3,000 \times g$ 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 and insulin was pooled over sampling time points by cow and period and stored at -20°C until analysis. Plasma AA, peptides, and AA metabolite concentrations were determined using an ultra-performance liquid chromatography-mass spectrometry system (Waters Acquity Ultra Performance LC system, Waters Corp., Milford, MA) as described by Haque et al. (2012). Plasma concentrations of glucose,

BHB, NEFA, TAG, urea, and insulin were analyzed by the Veterinary Diagnostic Laboratory (Utrecht University, the Netherlands) as described by van Knegsel et al. (2007).

Calculations and Statistical Analysis

Plasma concentrations of AA, peptides, and AA metabolites were averaged over the 5 sampling times. 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. Long-chain fatty acid concentrations were calculated on a molar basis as $3 \times \text{TAG} + \text{NEFA}$. Although it is a semi-essential AA for dairy cattle, Arg has been included in all calculations of total EAA. 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., 1993b), where $\text{MPF (L/h)} = [\text{milk Phe} + \text{Tyr output (}\mu\text{mol/h)}] / [\text{AV Phe} + \text{Tyr difference (}\mu\text{mol/L)}]$, 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 5, corresponding to the blood samples taken that day, using mean Phe and Tyr contents reported by Mephram (1987) and Lapierre et al. (2012). Uptakes (mmol/h) of metabolites across the mammary glands were calculated as the product of their plasma AV differences and MPF. Positive uptakes indicate a net removal from plasma, whereas negative values indicate net release from the mammary glands. Mammary metabolite clearances were calculated from the model of Hanigan et al. (1998), where $\text{clearance (L/h)} = (\text{AV difference} \times \text{MPF}) / \text{venous concentration}$. Mean milk protein AA composition reported by Mephram (1987) and Lapierre et al. (2012) was used to calculate AA uptake to output ratios (U:O). Mammary gland balance for glucose and LCFA were calculated according to estimations of Dijkstra et al. (1996) for glucose and LCFA, using milk protein, lactose, and fat yield from the afternoon milking on d 5 of infusion. The molecular weight of FA $\geq 16\text{C}$ in blood was calculated according to the molecular weight of $\geq 16\text{C}$ FA in milk (Nichols et al., 2019b), assuming that 50% of milk C16 originated from C16 FA in blood sequestered as preformed FA, and that 50% of milk C16 was synthesized de novo.

Variances in milk and milk component production, plasma constituent concentrations and AV differences, MPF, mammary metabolite uptakes and clearances, mammary metabolite U:O, and mammary metabolite balances were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The model contained main and interaction effects of infusion treat-

ment factors (GG, LG, and AA) 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 were acknowledged at $0.05 < P \leq 0.10$. Multiple comparisons between treatment means were made using the Tukey-Kramer method when $GG \times AA$ or $LG \times AA$ interactions were detected at $P \leq 0.10$.

RESULTS

Milk Production

Daily lactation performance and DMI have been presented by Nichols et al. (2019b). The present paper reports milk production expressed on an hourly basis from the afternoon milking on d 5 of infusion (Table 1). Infusing GG or LG at the HMP level did not affect milk production or composition differently than at the LMP level (no $GG \times AA$ or $LG \times AA$ interactions; $P > 0.10$). Yields of milk, protein, and lactose were unaffected by GG and LG ($P > 0.23$) and increased at the HMP level ($P < 0.01$). The HMP level increased milk protein content ($P < 0.01$) and decreased lactose content ($P < 0.01$). Milk fat yield tended to decrease in response to GG ($P = 0.10$), tended to increase in response to LG ($P = 0.09$), and increased at the HMP level ($P < 0.01$). Milk fat content tended to decrease in response to GG ($P = 0.06$) and increased in response to LG ($P = 0.02$). Milk urea content tended to decrease in response to GG ($P = 0.06$) and increased at the HMP level ($P = 0.01$).

Arterial Metabolite and Insulin Concentrations

Irrespective of MP level, arterial plasma concentration of glucose and insulin increased in response to GG ($P \leq 0.04$; Table 2) and concentration of BHB, NEFA, and LCFA decreased ($P \leq 0.03$). Plasma urea tended to be affected by a $GG \times AA$ interaction, whereby its concentration decreased with GG but only at the HMP level ($P = 0.08$). Regardless of MP level, arterial plasma concentration of total EAA, group 2 AA, and branched-chain AA (BCAA; $P < 0.01$) decreased in response to GG. A $GG \times AA$ interaction affected Leu concentration ($P = 0.05$), which decreased with GG but only at the HMP level. Infusion of GG decreased Arg, Ile, Lys, and Val concentrations ($P < 0.01$), regardless of MP level. A $GG \times AA$ interaction affected Ala, Asp, and Glu ($P \leq 0.04$), whereby GG infusion decreased Ala concentration at the LMP level, and GG infusion resulted in numerically lower Asp and Glu at the LMP level compared with the HMP level. Irrespec-

Table 1. Milk and component production of lactating dairy cows ($n = 6$) receiving abomasal infusions of saline (control; C), glucose (GG), and palm olein (LG) at low (LMP) and high (HMP) metabolizable protein levels for 5 d¹

Item	Treatment ²							P-value ³					
	LMP-C	LMP-GG	LMP-LG	HMP-C	HMP-GG	HMP-LG	SEM	GG	LG	AA	GG × AA	LG × AA	
Milk, kg/h	1.08	1.04	1.05	1.26	1.30	1.27	0.070	0.85	0.65	<0.01	0.16	0.47	
Protein, g/h	34.3	32.2	32.2	43.1	44.6	42.6	2.20	0.79	0.24	<0.01	0.11	0.49	
Protein, g/kg	31.9	31.2	30.8	34.2	34.4	33.5	0.61	0.65	0.12	<0.01	0.39	0.65	
Fat, g/h	50.0	42.6	53.4	55.5	53.8	60.8	3.14	0.10	0.09	<0.01	0.29	0.76	
Fat, g/kg	46.0	41.2	51.3	44.0	41.5	48.1	1.84	0.06	0.02	0.46	0.52	0.77	
Lactose, g/h	51.0	49.6	49.6	57.4	58.9	57.9	3.28	0.96	0.77	<0.01	0.35	0.53	
Lactose, g/kg	47.0	47.9	47.1	45.5	45.4	45.5	0.34	0.25	0.86	<0.01	0.19	0.98	
Urea, mg/dL	9.5	8.8	9.3	15.3	10.3	15.5	1.94	0.06	0.95	0.01	0.14	0.91	

¹Data are least squares means from the afternoon milking on d 5 of infusion.

²Abomasal infusion treatments: LMP-C, 0.9% saline; LMP-GG, 1,319 g/d of glucose; LMP-LG, 676 g/d of palm olein; HMP-C, 844 g/d of a complete EAA mixture in the same profile and amount as found in 1.5 kg of casein; HMP-GG, 1,319 g/d of glucose + 844 g/d of EAA; HMP-LG, 676 g/d of palm olein + 844 g/d of EAA. Infusions of LMP-GG, LMP-LG, and HMP-C supplied 20.5 MJ/d, and HMP-GG and HMP-LG supplied 41.0 MJ/d.

³GG = effect of energy from glucose; LG = effect of energy from fat; AA = effect of protein level.

tive of MP level, infusion of GG increased Gly and Ser ($P \leq 0.01$), decreased Orn ($P < 0.01$), and tended to decrease Cit and Pro ($P \leq 0.08$). Infusion of GG decreased carnosine concentration but only at the LMP level (GG $\times$ AA interaction; $P = 0.03$) and tended to decrease concentration of α -amino-*n*-butyric acid ($P = 0.09$) and α -amino-adipic acid ($P = 0.10$) irrespective of MP level.

Infusion of LG increased arterial plasma concentration of TAG and LCFA ($P < 0.01$) irrespective of MP level. Plasma urea concentration was numerically lower at the LMP level and numerically higher at the HMP level with LG infusion (tendency for LG $\times$ AA interaction; $P = 0.09$). Infusion of LG did not affect plasma concentration of any AA group or any individual EAA ($P > 0.39$). A LG $\times$ AA interaction tended to affect Pro concentration, which decreased only at the HMP level ($P = 0.09$). Infusion of LG tended to decrease concentrations of Asn ($P = 0.06$) and Tyr ($P = 0.07$). Concentration of 1 methyl-histidine was higher with LG infusion at the LMP level compared with the HMP level (LG $\times$ AA interaction; $P = 0.05$).

The HMP level increased arterial plasma concentrations of BHB, urea (dependent on GG and LG level), and concentrations of all EAA groups ($P \leq 0.01$). All individual EAA increased at the HMP level ($P < 0.01$). The HMP level decreased total NEAA concentration ($P < 0.01$), and individual concentrations of Ala (dependent on GG level), Asn, Gln, Gly, Pro (dependent on LG level), and Ser ($P \leq 0.05$). The HMP level increased Cit, Orn, and Tyr ($P \leq 0.01$) and tended to increase Cys ($P = 0.08$). The HMP level increased the concentration of α -amino-*n*-butyric acid, α -amino-adipic acid, carnosine (dependent on GG level), cystathionine, and phosphoserine ($P \leq 0.01$), and decreased 1 methyl-histidine (dependent on LG level), 3 methyl-histidine, and hydroxyproline ($P \leq 0.04$).

Mammary Metabolite AV Differences

Regardless of MP level, GG decreased the AV difference of BHB and TAG ($P < 0.01$) and tended to decrease that of LCFA ($P = 0.07$; Table 3). The AV differences of all EAA groups were affected by a GG $\times$ AA interaction, decreasing with GG only at the HMP level ($P \leq 0.05$). Individually, this interaction affected Met, Phe, Thr, Trp, and Val ($P \leq 0.05$) and tended to affect His ($P = 0.09$), Ile ($P = 0.10$), and Leu ($P = 0.08$). Infusion of GG decreased AV differences of Arg and Lys, irrespective of MP level ($P < 0.01$). The AV difference of total NEAA tended to be affected by a GG $\times$ AA interaction, decreasing with GG only at the HMP level ($P = 0.06$). Individually, this interaction affected Cit, Gln, Gly, and Pro ($P \leq 0.05$) and tended to

affect Ser ($P = 0.07$) and Tyr ($P = 0.09$). The AV difference of Glu was numerically decreased at the LMP level and increased at the HMP level (tendency for GG $\times$ AA interaction; $P = 0.06$). Regardless of MP level, GG decreased the AV differences of Asn and Orn ($P \leq 0.02$).

Regardless of MP level, LG increased the AV difference of TAG and LCFA ($P < 0.01$). Infusion of LG did not affect the AV difference of any AA group or individual AA ($P > 0.10$), except that of His, which tended to decrease ($P = 0.07$). The HMP level increased the AV difference of BHB ($P < 0.01$). The AV differences of all EAA groups increased at the HMP level (all dependent on GG level; $P \leq 0.01$). Individually, the AV differences of all EAA except Trp increased at the HMP level ($P \leq 0.03$), with increases for His, Ile, Leu, Met, Phe, Thr, and Val depending on GG level. The AV differences of Ala and Pro decreased (for Pro dependent on GG level; $P \leq 0.02$) and the AV difference of Gly tended to decrease (dependent on GG level; $P = 0.07$) at the HMP level.

Mammary Plasma Flow and Metabolite Uptake

Irrespective of MP level, infusion of GG increased MPF ($P = 0.02$; Table 4) and tended to increase mammary net glucose uptake ($P = 0.07$; Table 4). A GG $\times$ AA interaction tended to affect BHB uptake, which was numerically lower with GG only at the LMP level ($P = 0.10$). A GG $\times$ AA interaction tended to affect uptake of total EAA ($P = 0.09$) and group 2 AA ($P = 0.08$), whereby their uptake was numerically lower at the LMP level and not affected at the HMP level. A GG $\times$ AA interaction affected Arg and Lys ($P \leq 0.03$) and tended to affect Ile and Leu ($P \leq 0.08$), whereby their uptake was decreased with GG at the LMP level. Uptake of Trp was higher with GG at the LMP level (GG $\times$ AA interaction; $P = 0.03$). Irrespective of MP level, infusion of GG decreased uptake of His and Met ($P = 0.05$). A GG $\times$ AA interaction affected uptake of Glu, Orn, and Tyr, with Glu uptake increasing with GG only at the HMP level ($P = 0.02$), Orn uptake decreasing with GG only at the LMP level ($P = 0.04$), and Tyr uptake being numerically higher with GG at the HMP level ($P = 0.03$). A GG $\times$ AA interaction tended to affect uptake of Cit, which was numerically lower at the HMP level ($P = 0.07$).

Irrespective of MP level, LG infusion increased mammary net uptake of TAG and LCFA ($P < 0.01$) and had no effect on uptake of any AA group or individual AA, except Ser uptake, which tended to increase ($P = 0.07$). Mammary net uptake of BHB was affected by MP level but only in the presence of GG ($P = 0.02$). The HMP level increased mammary uptake of all EAA

Table 2. Arterial plasma concentrations of metabolites and insulin in lactating dairy cows (n = 6) receiving abomasal infusions of saline (control; C), glucose (GG), and palm olein (LG) at low (LMP) and high (HMP) metabolizable protein levels for 5 d

Item	Treatment ¹										P-value ²			
	LMP-C	LMP-GG	LMP-LG	HMP-C	HMP-GG	HMP-LG	SEM	GG	LG	AA	GG × AA	LG × AA	GG × LG	AA × LG
Glucose, mM	3.45	4.00	3.60	3.57	4.02	3.57	0.107	<0.01	0.41	0.92	0.58	0.41	<0.01	<0.01
BHB, mM	0.65	0.36	0.58	0.71	0.49	0.71	0.050	<0.01	0.37	<0.01	0.44	0.37	<0.01	0.44
Nonesterified fatty acids, μ M	162	62	152	110	65	180	36.5	0.03	0.35	0.62	0.39	0.22	0.03	0.39
Triacylglycerides, μ M	48	47	87	57	50	95	4.0	0.30	<0.01	0.15	0.53	0.99	<0.01	0.53
Long-chain fatty acids, μ M	307	202	412	280	215	465	38.0	0.02	<0.01	0.32	0.55	0.24	<0.01	0.55
Urea, mM	2.07 ^a	1.87 ^a	2.33 ^{ac}	3.10 ^b	2.35 ^{ac}	2.83 ^{bc}	0.194	<0.01	0.99	<0.01	0.08	0.09	<0.01	0.08
Insulin, μ IU/L	12.9	17.2	11.4	14.5	18.9	14.4	2.47	0.04	0.68	0.26	0.99	0.73	0.04	0.99
AA, μ M														
EAA ³	843	566	864	2,036	1,713	2,007	112.8	<0.01	0.98	<0.01	0.53	0.84	<0.01	0.53
Group 1 ⁴	182	161	174	463	459	438	26.4	0.46	0.57	<0.01	0.83	0.84	<0.01	0.83
Group 2 ⁵	710	449	733	1,634	1,319	1,622	93.8	<0.01	0.91	<0.01	0.45	0.85	<0.01	0.45
BCAA ⁶	448	251	480	1,106	735	1,139	70.1	<0.01	0.82	<0.01	0.14	0.79	<0.01	0.14
NEAA ⁷	1,599	1,570	1,676	1,292	1,407	1,143	84.4	0.42	0.69	<0.01	0.22	0.13	<0.01	0.22
TAA ⁸	2,442	2,136	2,540	3,328	3,120	3,151	161.2	0.05	0.86	<0.01	0.86	0.35	<0.01	0.86
Arg	76	51	78	131	112	122	6.0	<0.01	0.41	<0.01	0.61	0.27	<0.01	0.61
His	22	15	19	95	103	85	5.2	0.96	0.40	<0.01	0.22	0.73	<0.01	0.22
Ile	130	66	141	231	148	231	13.9	<0.01	0.98	<0.01	0.45	0.48	<0.01	0.45
Leu	110 ^a	64 ^a	117 ^{ac}	312 ^b	200 ^c	317 ^b	21.2	<0.01	0.93	<0.01	0.05	0.77	<0.01	0.05
Lys	62	39	66	158	138	149	9.5	<0.01	0.60	<0.01	0.87	0.33	<0.01	0.87
Met	21	17	21	95	99	92	5.5	0.81	0.95	<0.01	0.57	0.95	<0.01	0.57
Phe	51	46	52	158	141	158	11.1	0.13	0.91	<0.01	0.34	0.96	<0.01	0.34
Thr	123	108	110	239	334	212	26.2	0.33	0.97	<0.01	0.13	0.64	<0.01	0.13
Trp	40	39	39	55	52	50	4.0	0.41	0.41	<0.01	0.63	0.50	<0.01	0.63
Val	208	121	223	564	387	591	36.6	<0.01	0.71	<0.01	0.14	0.95	<0.01	0.14
Ala	244 ^{ab}	206 ^b	273 ^{ab}	201 ^b	228 ^{ab}	196 ^b	17.1	0.83	0.26	0.03	<0.01	0.14	<0.01	<0.01
Asn	57	47	55	50	49	40	3.7	0.14	0.06	0.05	0.11	0.21	<0.01	0.11
Asp	10.5	6.4	10.4	7.5	8.5	7.1	1.44	0.22	0.88	0.67	0.04	0.96	<0.01	0.04
Cit	75	63	75	97	86	97	11.6	0.07	0.88	<0.01	0.92	0.85	<0.01	0.92
Cys	7.5	5.0	4.9	9.5	9.2	7.8	1.76	0.47	0.30	0.08	0.62	0.76	<0.01	0.62
Gln	395	359	393	270	272	220	29.1	0.62	0.25	<0.01	0.31	0.30	<0.01	0.31
Glu	54 ^a	45 ^{ab}	54 ^a	45 ^{ab}	49 ^{ab}	42 ^b	2.9	0.57	0.65	0.27	0.03	0.65	<0.01	0.03
Gly	470	566	526	329	407	297	29.8	<0.01	0.56	<0.01	0.89	0.17	<0.01	0.89
Orn	34	22	32	57	47	48	3.6	<0.01	0.42	<0.01	0.78	0.18	<0.01	0.78
Pro	90 ^a	75 ^{ab}	91 ^a	78 ^{ab}	76 ^{ab}	64 ^b	6.0	0.08	0.15	<0.01	0.11	0.09	<0.01	0.11
Ser	114	132	120	90	111	73	5.8	0.01	0.74	<0.01	0.88	0.20	<0.01	0.88
Tyr	49	44	43	60	65	52	3.8	0.89	0.07	<0.01	0.13	0.83	<0.01	0.13
Other AA, peptides, and AA metabolites, μ M														
1 Methyl-histidine	4.2 ^{ab}	4.5 ^a	5.2 ^a	3.3 ^b	3.4 ^b	3.2 ^b	0.36	0.55	0.09	<0.01	0.72	0.05	<0.01	0.72
3 Methyl-histidine	5.4	4.8	5.8	4.8	4.1	4.1	0.39	0.15	0.59	0.01	0.93	0.13	<0.01	0.93
α -Amino- <i>n</i> -butyric acid	29	18	27	46	40	45	6.0	0.09	0.74	<0.01	0.67	0.97	<0.01	0.67
α -Amino-adipic acid	3.1	2.0	3.2	6.3	5.9	6.9	0.43	0.10	0.54	<0.01	0.53	0.71	<0.01	0.53
β -Alanine	3.9	4.8	3.4	4.3	4.6	3.4	0.55	0.31	0.31	0.99	0.66	0.83	<0.01	0.66
Carnosine	13 ^{abc}	10 ^c	11 ^{bc}	16 ^{ab}	17 ^a	15 ^{ab}	1.5	0.42	0.13	<0.01	0.03	0.69	<0.01	0.03
Cystathionine	1.9	1.4	1.6	3.2	3.2	3.0	0.24	0.19	0.11	<0.01	0.14	0.96	<0.01	0.14
Hydroxylysine	0.8	1.1	1.0	0.7	0.8	0.8	0.34	0.41	0.62	0.36	0.66	0.75	<0.01	0.36

Continued

Table 2 (Continued). Arterial plasma concentrations of metabolites and insulin in lactating dairy cows (n = 6) receiving abomasal infusions of saline (control; C), glucose (GG), and palm olein (LG) at low (LMP) and high (HMP) metabolizable protein levels for 5 d

Item	Treatment ¹						P-value ²					
	LMP-C	LMP-GG	LMP-LG	HMP-C	HMP-GG	HMP-LG	SEM	GG	LG	AA	GG × AA	LG × AA
Hydroxyproline	15	16	15	12	15	11	1.1	0.17	0.59	0.04	0.38	0.68
Phosphoserine	2.3	1.8	2.3	3.4	4.7	3.5	0.67	0.82	0.42	0.01	0.60	0.44
Taurine	32	33	30	39	39	28	3.9	0.93	0.13	0.55	0.99	0.32

^aMeans within a row with no common superscripts differ ($P < 0.05$).
¹Abomasal infusion treatments: LMP-C, 0.9% saline; LMP-GG, 1,319 g/d of glucose; LMP-LG, 676 g/d of palm olein; HMP-C, 844 g/d of a complete EAA mixture in the same profile and amount as found in 1.5 kg of casein; HMP-GG, 1,319 g/d of glucose + 844 g/d of EAA; HMP-LG, 676 g/d of palm olein + 844 g/d of EAA. Infusions of LMP-GG, LMP-LG, and HMP-C supplied 20.5 MJ/d, and HMP-GG and HMP-LG supplied 41.0 MJ/d.
²GG = effect of energy from glucose; LG = effect of energy from fat; AA = effect of protein level.
³EAA = Arg (considered semi-essential), His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.
⁴Group 1 = His, Met, Phe+Tyr, Trp.
⁵Group 2 = Arg, Ile, Leu, Lys, Thr, Val.
⁶BCAA (branched-chain AA) = Ile, Leu, Val.
⁷NEAA = Ala, Asn, Asp, Cit, Cys, Gln, Glu, Gly, Orn, Pro, Ser, Tyr.
⁸TAA (total AA) = EAA + NEAA.
⁹Other N derivatives measured with ultra-performance liquid chromatography-mass spectrometry showing a plasma concentration higher than the limit of quantification.

groups ($P < 0.01$). The HMP level increased individual uptake of all EAA ($P < 0.01$), with the exception of Trp, which was unaffected, while uptakes of Arg and Lys were dependent on GG level. Mammary net uptake of total NEAA decreased at the HMP level ($P = 0.03$). Individually, the HMP level increased Tyr uptake ($P = 0.01$; dependent on GG), tended to increase Asn uptake ($P = 0.08$), and decreased uptake of Ala, Gly, and Pro ($P \leq 0.04$).

Mammary Metabolite Clearance

Infusion of GG had no effect on mammary clearance of glucose, BHB, NEFA, TAG, or LCFA, irrespective of MP level ($P \geq 0.11$; Table 5). Total EAA clearance was affected ($P = 0.04$) and group 2 AA clearance tended to be affected ($P = 0.10$) by a GG × AA interaction, with both increasing in response to GG only at the LMP level. Clearance of total BCAA increased in response to GG regardless of MP level ($P < 0.01$). Individually, a GG × AA interaction affected Leu and Trp ($P \leq 0.05$) and tended to affect Arg and Val ($P \leq 0.08$), with clearances increasing in response to GG only at the LMP level. Clearance of Ile increased ($P < 0.01$) and clearance of Lys tended to increase ($P = 0.06$) in response to GG, regardless of MP level. A GG × AA interaction affected Cit ($P = 0.05$) and tended to affect Cys ($P = 0.07$), with their clearances increasing in response to GG only at the LMP level. Infusion of GG increased Glu clearance regardless of MP level ($P = 0.04$).

Infusion of LG increased clearance of TAG ($P = 0.01$) and tended to increase clearance of LCFA ($P = 0.06$), regardless of MP level. Irrespective of MP level, LG did not affect clearance of any AA group or any individual EAA. A LG × AA interaction affected Asp ($P = 0.04$) and tended to affect Asn ($P = 0.06$), with clearances increasing with LG only at the HMP level. Clearance of Ser increased in response to LG regardless of MP level ($P < 0.01$). The HMP level tended to increase mammary clearance of BHB ($P = 0.10$). Clearance of all EAA groups decreased at the HMP level ($P < 0.01$). Clearance of total EAA was affected by a GG × AA interaction ($P = 0.04$), decreasing more at the HMP level relative to the LMP level in the presence of GG. Clearances of all individual EAA decreased at the HMP level ($P \leq 0.05$), except clearances of Trp and Val, which were affected by MP level in the presence of GG ($P \leq 0.07$), and Arg and Leu, which decreased more at the HMP level relative to the LMP level in the presence of GG ($P \leq 0.08$). The HMP level increased clearance of Asn (dependent on LG), Gln, and Ser ($P < 0.01$); tended to increase clearance of Asp ($P = 0.08$; depen-

Table 3. Mammary gland arteriovenous differences of metabolites in lactating dairy cows (n = 6) receiving abomasal infusions of saline (control; C), glucose (GG), and palm olein (LG) at low (LMP) and high (HMP) metabolizable protein levels for 5 d

Item	Treatment ¹							P-value ²				
	LMP-C	LMP-GG	LMP-LG	HMP-C	HMP-GG	HMP-LG	SEM	GG	LG	AA	GG × AA	LG × AA
Glucose, mM	0.75	0.73	0.80	0.83	0.80	0.73	0.065	0.78	0.78	0.99	0.85	0.27
BHB, mM	0.20	0.10	0.16	0.27	0.18	0.25	0.023	<0.01	0.18	<0.01	0.87	0.77
Nonesterified fatty acids, μM	12	17	-2	23	12	40	20.5	0.87	0.93	0.36	0.68	0.45
Triacylglycerides, μM	30	22	62	37	20	68	3.7	<0.01	<0.01	0.46	0.22	0.99
Long-chain fatty acids, μM	102	82	183	133	72	245	21.7	0.07	<0.01	0.24	0.34	0.49
AA, μM												
EAA ³	304 ^a	239 ^a	303 ^a	607 ^b	394 ^{ac}	520 ^{bc}	44.9	<0.01	0.22	<0.01	0.03	0.24
Group 1 ⁴	73 ^{ab}	68 ^b	70 ^b	144 ^c	90 ^{ab}	113 ^{ac}	10.3	0.01	0.11	0.01	0.02	0.17
Group 2 ⁵	252 ^a	190 ^a	252 ^a	493 ^b	324 ^{ac}	431 ^{bc}	37.9	<0.01	0.29	<0.01	0.05	0.28
BCAA ⁶	147 ^a	106 ^a	145 ^a	315 ^b	193 ^{ac}	269 ^{bc}	27.0	<0.01	0.24	<0.01	0.05	0.29
NEAA ⁷	252	286	287	311	205	266	40.5	0.29	0.90	0.14	0.06	0.28
TAA ⁸	556 ^a	525 ^a	590 ^a	918 ^b	599 ^a	786 ^{ab}	76.0	0.01	0.44	0.08	0.02	0.19
Arg	35	28	36	53	39	49	3.5	<0.01	0.47	<0.01	0.17	0.42
His	14 ^{ab}	10 ^b	12 ^{ab}	28 ^c	17 ^{ab}	20 ^{ac}	2.2	<0.01	0.07	0.01	0.09	0.18
Ile	41	28	39	78	51	68	6.2	<0.01	0.15	<0.01	0.10	0.35
Leu	55 ^{ab}	39 ^b	54 ^{ab}	114 ^c	75 ^{ad}	99 ^{cd}	8.6	<0.01	0.23	<0.01	0.08	0.30
Lys	39	26	41	69	55	66	4.9	<0.01	0.83	<0.01	0.72	0.33
Met	13 ^{ab}	10 ^b	13 ^{ab}	27 ^c	15 ^{ab}	21 ^{ac}	2.2	<0.01	0.17	0.01	0.05	0.18
Phe	21 ^a	21 ^a	21 ^a	49 ^b	31 ^{ac}	39 ^{bc}	3.4	0.01	0.16	<0.01	0.02	0.21
Thr	31 ^a	30 ^a	31 ^a	56 ^b	37 ^{ab}	48 ^{ab}	5.5	0.02	0.58	0.03	0.03	0.55
Trp	5	9	5	11	7	8	1.5	0.91	0.39	0.85	0.02	0.43
Val	51 ^{ab}	39 ^b	52 ^{ab}	123 ^c	67 ^{ab}	101 ^{ac}	12.9	<0.01	0.32	<0.01	0.04	0.29
Ala	29	41	37	18	11	19	9.4	0.88	0.55	<0.01	0.22	0.70
Asn	14	12	13	19	13	17	1.9	0.02	0.38	0.29	0.15	0.43
Asp	0.5	0.2	0.8	0.2	1.0	0.9	0.55	0.82	0.26	0.40	0.44	0.63
Cit	4	8	5	11	3	8	2.6	0.17	0.64	0.42	0.01	0.42
Cys	0.7	1.3	0.6	1.6	0.7	1.2	0.52	0.85	0.48	0.99	0.18	0.65
Gln	76	77	84	101	62	81	12.0	0.04	0.54	0.29	0.04	0.18
Glu	37	31	37	32	36	30	3.0	0.77	0.69	0.72	0.06	0.72
Gly	26	49	35	39	19	29	10.3	0.99	0.95	0.07	0.04	0.38
Orn	17	12	17	20	14	19	2.0	<0.01	0.52	0.33	0.90	0.58
Pro	15	16	16	16	9	13	2.4	0.12	0.65	0.02	0.05	0.30
Ser	12	21	22	24	18	25	4.2	0.72	0.18	0.93	0.07	0.26
Tyr	20 ^a	19 ^a	20 ^a	30 ^b	21 ^a	24 ^{ab}	2.5	0.02	0.11	0.21	0.09	0.19

^{a-d}Means within a row with no common superscripts differ ($P < 0.05$).¹Abomasal infusion treatments: LMP-C, 0.9% saline; LMP-GG, 1,319 g/d of glucose; LMP-LG, 676 g/d of palm olein; HMP-C, 844 g/d of a complete EAA mixture in the same profile and amount as found in 1.5 kg of casein; HMP-GG, 1,319 g/d of glucose + 844 g/d of EAA; HMP-LG, 676 g/d of palm olein + 844 g/d of EAA. Infusions of LMP-GG, LMP-LG, and HMP-C supplied 20.5 MJ/d, and HMP-GG and HMP-LG supplied 41.0 MJ/d.²GG = effect of energy from glucose; LG = effect of energy from fat; AA = effect of protein level.³EAA = Arg (considered semi-essential), His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.⁴Group 1 = His, Met, Phe+Tyr, Trp.⁵Group 2 = Arg, Ile, Leu, Lys, Thr, Val.⁶BCAA (branched-chain AA) = Ile, Leu, Val.⁷NEAA = Ala, Asn, Asp, Cit, Cys, Gln, Glu, Gly, Orn, Pro, Ser, Tyr.⁸TAA (total AA) = EAA + NEAA.

Table 4. Whole-mammary gland plasma flow and net uptakes of metabolites in lactating dairy cows (n = 6) receiving abomasal infusions of saline (control; C), glucose (GG), and palm olein (LG) at low (LMP) and high (HMP) metabolizable protein levels for 5 d

Item	Treatment ¹										P-value ²			
	LMP-C	LMP-GG	LMP-LG	HMP-C	HMP-GG	HMP-LG	SEM	GG	LG	AA	GG × AA	LG × AA		
Plasma flow, L/h	489	520	468	337	507	394	56.0	0.02	0.66	0.31	0.11	0.35		
Net mammary uptake, mmol/h														
Glucose	366.9	369.6	370.6	276.3	411.4	292.9	43.51	0.07	0.68	0.64	0.14	0.99		
BHB	90.8	51.7	75.0	87.3	87.3	94.2	10.55	0.10	0.70	0.02	0.10	0.33		
Nonesterified fatty acids	8.3	8.2	-3.9	11.9	7.6	12.1	9.84	0.84	0.51	0.39	0.85	0.50		
Triacylglycerides	14.4	10.8	28.8	12.1	12.4	27.1	2.65	0.49	<0.01	0.96	0.37	0.88		
Long-chain fatty acids	51.6	40.4	82.4	48.3	45.2	93.5	10.58	0.42	<0.01	0.29	0.56	0.36		
AA														
EAA ³	141.8 ^a	114.9 ^a	135.0 ^a	191.5 ^b	197.5 ^b	199.4 ^b	14.43	0.28	0.96	<0.01	0.09	0.44		
Group 1 ⁴	33.9	31.6	31.6	44.1	43.8	42.8	2.40	0.33	0.19	<0.01	0.44	0.68		
Group 2 ⁵	117.3 ^a	91.8 ^a	112.1 ^a	156.7 ^b	164.0 ^b	165.8 ^b	12.87	0.31	0.83	<0.01	0.08	0.43		
BCAA ⁶	68.4	51.3	63.9	99.8	97.7	102.0	8.08	0.13	0.85	<0.01	0.24	0.59		
NEAA ⁷	118.8	127.7	128.0	96.1	102.4	106.1	13.90	0.48	0.37	0.03	0.91	0.97		
TAA ⁸	260.7	242.5	263.0	287.6	299.9	305.5	26.11	0.86	0.54	0.01	0.36	0.64		
Arg	16.4 ^{abc}	13.6 ^c	15.9 ^{bc}	16.9 ^{abcd}	19.7 ^d	19.0 ^{ad}	1.55	0.98	0.41	<0.01	0.01	0.18		
His	6.4	4.9	5.6	8.3	8.1	7.6	0.59	0.05	0.11	<0.01	0.16	0.82		
Ile	19.0 ^a	13.5 ^a	16.9 ^a	25.0 ^b	25.8 ^b	25.9 ^b	1.95	0.16	0.72	<0.01	0.06	0.36		
Leu	25.7 ^a	19.3 ^a	24.1 ^a	36.6 ^b	38.0 ^b	37.9 ^b	2.86	0.26	0.95	<0.01	0.08	0.51		
Lys	18.4 ^{ab}	13.4 ^b	18.5 ^{ab}	22.6 ^{ac}	28.1 ^c	26.2 ^c	2.62	0.89	0.36	<0.01	0.01	0.39		
Met	5.9	4.7	5.7	8.2	7.6	8.0	0.62	0.05	0.63	<0.01	0.52	0.96		
Phe	9.9	9.6	9.4	15.0	14.9	14.6	0.77	0.68	0.40	<0.01	0.89	0.88		
Thr	14.1	13.5	13.8	17.4	18.5	18.6	1.87	0.86	0.74	<0.01	0.56	0.60		
Trp	2.4	3.8	2.3	3.3	3.0	3.2	0.37	0.12	0.75	0.83	0.03	0.98		
Val	23.8	18.6	22.9	38.3	33.8	38.2	3.63	0.11	0.87	<0.01	0.90	0.89		
Ala	14.1	18.8	16.9	5.6	5.6	8.8	3.76	0.44	0.33	<0.01	0.44	0.96		
Asn	6.3	5.1	5.9	6.1	6.3	6.7	0.71	0.38	0.91	0.08	0.20	0.39		
Asp	0.3	0.3	0.3	0.1	0.5	0.4	0.24	0.31	0.41	0.46	0.28	0.53		
Cit	1.8	3.0	2.1	2.8	1.1	2.7	0.85	0.70	0.94	0.44	0.07	0.81		
Cys	0.3	0.5	0.2	0.5	0.3	0.5	0.17	0.87	0.73	0.74	0.27	0.99		
Gln	34.8	33.9	37.7	30.8	31.2	31.9	3.87	0.94	0.51	0.17	0.83	0.76		
Glu	17.9 ^a	16.4 ^{ab}	16.9 ^{ab}	11.0 ^c	17.8 ^a	12.1 ^{bc}	2.11	0.11	0.98	0.29	0.02	0.51		
Gly	12.5	20.1	15.0	10.8	8.6	11.3	3.65	0.44	0.66	0.04	0.17	0.77		
Orn	8.4 ^a	6.0 ^b	7.7 ^{ab}	6.6 ^{ab}	7.4 ^{ab}	7.4 ^{ab}	0.83	0.31	0.89	0.47	0.04	0.32		
Pro	7.0	7.1	7.0	4.9	4.4	5.1	0.94	0.76	0.88	<0.01	0.66	0.89		
Ser	6.2	8.2	9.5	7.7	8.9	10.1	1.86	0.30	0.07	0.67	0.78	0.77		
Tyr	9.4	8.5	8.8	9.2	10.2	9.3	0.67	0.91	0.47	0.01	0.03	0.39		

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

¹Abomasal infusion treatments: LMP-C, 0.9% saline; LMP-GG, 1,319 g/d of glucose; LMP-LG, 676 g/d of palm olein; HMP-C, 844 g/d of a complete EAA mixture in the same profile and amount as found in 1.5 kg of casein; HMP-GG, 1,319 g/d of glucose + 844 g/d of EAA; HMP-LG, 676 g/d of palm olein + 844 g/d of EAA. Infusions of LMP-GG, LMP-LG, and HMP-C supplied 20.5 MJ/d, and HMP-GG and HMP-LG supplied 41.0 MJ/d.

²GG = effect of energy from glucose; LG = effect of energy from fat; AA = effect of protein level.

³EAA = Arg (considered semi-essential), His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.

⁴Group 1 = His, Met, Phe+Trp, Trp.

⁵Group 2 = Arg, Ile, Leu, Lys, Thr, Val.

⁶BCAA (branched-chain AA) = Ile, Leu, Val.

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

⁸TAA (total AA) = EAA + NEAA.

Table 5. Mammary clearances (L/h) of metabolites in lactating dairy cows (n = 6) receiving abomasal infusions of saline (control; C), glucose (GG), and palm olein (LG) at low (LMP) and high (HMP) metabolizable protein levels for 5 d

Item	Treatment ¹							P-value ²					
	LMP-C	LMP-GG	LMP-LG	HMP-LG	HMP-C	HMP-GG	HMP-LG	SEM	GG	LG	AA	GG × AA	LG × AA
Glucose	132	113	136	101	134 ^c	156 ^c	138 ^c	20.3	<0.01	0.59	<0.01	0.04	0.45
BHB	205	197	190	202	145	127	137	19.8	0.85	0.40	<0.01	0.14	0.74
Nonesterified fatty acids	36	148	—44	82	137 ^b	171 ^{bd}	144 ^b	25.0	<0.01	0.72	<0.01	0.10	0.49
	828	498	1,282	641	226	184	122	29.9	<0.01	0.42	<0.01	0.11	0.50
Triacylglycerides	287	343	384	298	98	89	123	13.9	0.92	0.24	0.41	0.38	0.29
Long-chain fatty acids					122	134	132	13.9	0.47	0.74	0.13	0.68	0.34
	AA				283 ^{ab}	276 ^{ab}	276 ^{ab}	52.5	<0.01	0.66	<0.01	0.08	0.23
EAA ³	268 ^a	360 ^b	247 ^a	134 ^c	119	100	125	177.3	0.56	0.92	<0.01	0.43	0.91
Group 1 ⁴	322	345	306	145	216 ^b	283 ^{ab}	276 ^{ab}	52.5	<0.01	0.66	<0.01	0.08	0.23
Group 2 ⁵	265 ^a	369 ^c	243 ^{ad}	137 ^b	127	100	125	177.3	0.56	0.92	<0.01	0.43	0.91
BCAA ⁶	238	377	202	126	163	268	164	43.6	<0.01	0.52	0.05	0.39	0.50
NEAA ⁷	90	101	92	98	188 ^b	318 ^{ab}	182 ^b	55.8	<0.01	0.30	<0.01	0.05	0.36
TAA ⁸	140	152	134	119	251	361	330	108.4	0.06	0.90	<0.01	0.53	0.43
Arg	422 ^a	620 ^c	393 ^a	208 ^{ab}	120	98	115	53.7	0.55	0.94	<0.01	0.31	0.98
His	995	1,183	1,024	995	151	147	129	24.5	0.47	0.11	<0.01	0.37	0.60
Ile	231	398	183	163	97	67	117	38.8	0.94	0.14	<0.01	0.38	0.33
Leu	500 ^a	824 ^c	407 ^a	188 ^b	78 ^a	74 ^a	79 ^{ab}	11.7	0.02	0.99	0.07	0.01	0.92
Lys	862	1,079	805	251	86 ^a	108 ^a	82 ^a	20.6	<0.01	0.52	<0.01	0.07	0.68
Met	672	757	669	120	208 ^{ab}	186 ^a	291 ^b	26.4	0.72	0.09	<0.01	0.53	0.06
Phe	351	383	309	151	80.5	80.5	167.0	61.15	0.59	0.20	0.08	0.26	0.04
Thr	164	200	259	97	31	17	30	10.5	0.47	0.84	0.11	0.05	0.73
Trp	69 ^a	129 ^b	68 ^a	78 ^a	69	70	59	21.2	0.06	0.67	0.22	0.07	0.39
Val	157 ^a	242 ^b	139 ^a	86 ^a	197	168	240	27.1	0.87	0.23	<0.01	0.28	0.50
Ala	70	115	79	33	908	1,313	1,086	182.3	0.04	0.73	0.44	0.12	0.19
Asn	147 ^a	153 ^a	142 ^a	208 ^{ab}	38	24	43	9.0	0.80	0.77	0.85	0.16	0.81
Asp	67.9	38.0	28.3	−3.2	179	238	260	63.9	0.14	0.29	<0.01	0.89	0.43
Cit	23	52	29	31	79	67	98	17.1	0.53	0.52	0.05	0.14	0.45
Cys	37	119	65	69	118	106	219	28.2	0.88	0.01	<0.01	0.69	0.12
Gln	109	130	121	197	335	243	397	54.8	0.36	0.37	0.31	0.35	0.72
Glu	1,136	1,201	1,031	908									
Gly	29	40	30	38									
Orn	535	607	547	179									
Pro	97	126	95	79									
Ser	72	77	101	118									
Tyr	356	358	383	335									

^{a-d}Means within a row with no common superscripts differ ($P < 0.05$).¹Abomasal infusion treatments: LMP-C, 0.9% saline; LMP-GG, 1,319 g/d of glucose; LMP-LG, 676 g/d of palm olein; HMP-C, 844 g/d of a complete EAA mixture in the same profile and amount as found in 1.5 kg of casein; HMP-GG, 1,319 g/d of glucose + 844 g/d of EAA; HMP-LG, 676 g/d of palm olein + 844 g/d of EAA. Infusions of LMP-GG, LMP-LG, and HMP-C supplied 20.5 MJ/d, and HMP-GG and HMP-LG supplied 41.0 MJ/d.²GG = effect of energy from glucose; LG = effect of energy from fat; AA = effect of protein level.³EAA = Arg (considered semi-essential), His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.⁴Group 1 = His, Met, Phe+Tyr, Trp.⁵Group 2 = Arg, Ile, Leu, Lys, Thr, Val.⁶BCAA (branched-chain AA) = Ile, Leu, Val.⁷NEAA = Ala, Asn, Asp, Cit, Cys, Gln, Glu, Gly, Orn, Pro, Ser, Tyr.⁸TAA (total AA) = EAA + NEAA.

dent on LG); and decreased clearance of Ala, Orn, and Pro ($P \leq 0.05$).

Mammary AA U:O

Irrespective of MP level, GG tended to decrease the U:O of total BCAA ($P = 0.09$; Table 6). A GG $\times$ AA interaction affected U:O of Arg and Lys, which numerically decreased with GG at the LMP level and increased with GG at the HMP level ($P \leq 0.03$); affected U:O of Trp, which increased with GG but only at the LMP level ($P = 0.01$); and tended to affect U:O of Ile, which decreased with GG but only at the LMP level ($P = 0.08$). The U:O of His and Met decreased ($P \leq 0.03$) and U:O of Val tended to decrease ($P = 0.08$) in response to GG, regardless of MP level. A GG $\times$ AA interaction tended to affect Glu ($P = 0.08$) and Gly ($P = 0.09$), with GG numerically increasing U:O of Glu at the HMP level and numerically increasing U:O of Gly at the LMP level and decreasing it at the HMP level.

Regardless of MP level, infusion of LG had no effect on U:O of any AA group or individual AA, except for U:O of Ser, which increased ($P = 0.04$). The HMP level increased U:O of total EAA, group 2 AA, and BCAA ($P < 0.01$). In the presence of GG, the U:O of Ile and Lys increased at the HMP level compared with the LMP level ($P \leq 0.02$). The U:O of Leu, Phe, and Val increased ($P \leq 0.01$) and U:O of His ($P = 0.07$) and Met ($P = 0.08$) tended to increase at the HMP level. In the presence of GG, the U:O of Trp decreased at the HMP level compared with the LMP level ($P = 0.03$). The HMP level decreased the U:O of total NEAA ($P < 0.01$). Individually, U:O of all NEAA decreased at the HMP level ($P < 0.01$), except Asn, which tended to decrease ($P = 0.07$), and Asp, Cys, and Ser, which were unaffected ($P > 0.10$).

Mammary Glucose and LCFA Balance

Lactose output as a proportion of mammary glucose uptake decreased in response to GG regardless of MP level ($P = 0.03$) and increased at the HMP level ($P = 0.03$; Table 7). The calculated amount of glucose required for milk fat synthesis tended to decrease in response to GG ($P = 0.10$) and tended to increase in response to LG ($P = 0.09$). The calculated glucose required for lactose and milk fat synthesis increased at the HMP level ($P \leq 0.01$). Net glucose uptake by the mammary gland did not cover calculated glucose requirements for lactose and fat synthesis on any treatment, with the exception of LMP-GG. Infusion of GG reduced the deficit ($P = 0.03$), while the HMP level increased it ($P = 0.02$). Output of LCFA in milk decreased in response to GG and increased in response to

LG ($P < 0.01$). Net mammary LCFA uptake did not equilibrate with output in milk on any treatment, but infusion of LG reduced the deficit ($P = 0.04$).

DISCUSSION

The current results complement previously reported daily lactation performance, milk FA composition, and energy and N balance from the same study (Nichols et al., 2019b). Notably, GG reduced milk energy output and increased body energy retention, whereas LG increased milk energy output and did not affect body energy retention. Body N mobilization occurred with LMP-GG but N retention occurred with HMP-GG, and GG improved milk N efficiency regardless of protein level. Body N retention was positive with LG infusion, and LG had no effect on milk N efficiency (Nichols et al., 2019b). Considering the milk output and whole-body energy and N balance responses observed in this experiment, we investigated mammary gland metabolism in response to glucose and palm olein supplied at low and high MP levels in the present work.

Milk Protein Yield and AA Catabolism Increased at the HMP Level

The objective of EAA infusion in the current study was to create low and high MP levels over which to compare the effects of GG and LG. As expected, the HMP level increased the arterial concentration of total EAA to 2.5 times that of the LMP level and increased milk protein yield 32%, in line with previous reports of postprandial EAA infusions lasting 5 to 14 d (Doepel and Lapierre, 2010; Doelman et al., 2015; Nichols et al., 2016). In agreement with our hypothesis, mammary net uptake of all EAA groups increased at the HMP level, but MPF was not affected and mammary clearance decreased. Decreased mammary clearance of EAA is consistent with surplus EAA supply for milk protein synthesis. Surplus EAA become available for extramammary metabolism, which is in line with increased body N retention at the HMP level (Nichols et al., 2019b). The U:O of total group 1 AA (His, Met, Phe+Tyr, Trp) was not affected by MP level, while U:O of group 2 AA (Arg, Ile, Leu, Lys, Val, Thr) and NEAA increased and decreased, respectively, at the HMP level. This dynamic of mammary gland AA utilization during EAA supplementation, whereby group 2 AA mammary uptake and intramammary catabolism increases while NEAA uptake decreases, agrees with the observations of Nichols et al. (2016) during 5-d abomasal infusion of the same EAA profile and dose.

The average U:O of Phe at the HMP level was 1.23, which is relatively greater than the average U:O of the

Table 6. Mammary gland AA uptake to milk output ratios in lactating dairy cows (n = 6) receiving abomasal infusions of saline (control; C), glucose (GG), and palm olein (LG) at low (LMP) and high (HMP) metabolizable protein levels for 5 d

Item	Treatment ¹					P-value ²							
	LMP-C	LMP-GG	LMP-LG	HMP-C	HMP-LG	SEM	GG	LG	AA	GG × AA	LG × AA		
EAA ³	1.19	1.02*	1.21	1.27	1.27	0.061	0.18	0.44	<0.01	0.17	0.63		
Group 1 ⁴	1.01*	1.00*	1.00*	1.04	1.03*	0.018	0.16	0.58	0.46	0.32	0.81		
Group 2 ⁵	1.24	1.02*	1.26	1.30	1.32	0.075	0.21	0.41	0.01	0.13	0.60		
BCAA ⁶	1.21	0.96*	1.20	1.38	1.32	0.087	0.09	0.74	<0.01	0.32	0.72		
NEAA ⁷	0.67	0.78	0.77	0.42	0.44	0.058	0.27	0.20	<0.01	0.38	0.62		
TAA ⁸	0.89	0.88	0.96*	0.78	0.79	0.049	0.99	0.18	0.03	0.86	0.94		
TAA-N ⁹	0.94*	0.90†	1.00*	0.85	0.86	0.049	0.79	0.20	0.19	0.66	0.97		
Arg	2.53 ^{ab}	2.21 ^{bc}	2.59 ^a	2.04 ^c	2.32 ^{abc}	0.116	0.87	0.11	0.54	0.01	0.29		
His	1.11*	0.91	1.04*	1.16	1.08*	0.061	0.03	0.26	0.07	0.31	0.99		
Ile	1.33 ^{ab}	1.00 ^{b*}	1.27 ^{ab}	1.37 ^a	1.38 ^a	0.086	0.09	0.91	0.01	0.08	0.47		
Leu	1.07*	0.84	1.07*	1.19	1.21	0.068	0.18	0.62	<0.01	0.13	0.64		
Lys	0.98 ^{ab*}	0.75 ^b	1.06 ^{ab*}	0.95 ^{ab*}	1.16 ^a	0.090	0.93	0.20	0.02	0.03	0.66		
Met	0.98*	0.83	1.00*	1.07*	0.96*	0.053	0.02	0.84	0.08	0.74	0.85		
Phe	1.03*	1.07	1.04*	1.25	1.19	0.031	0.74	0.98	<0.01	0.13	0.71		
Thr	1.08*	1.10*	1.12*	1.05*	1.07*	0.092	0.86	0.44	0.99	0.97	0.72		
Trp	0.93 ^{ab*}	1.62 ^b	0.96 ^{a*}	1.05 ^{ab*}	0.93 ^{a*}	0.139	0.05	0.99	0.03	0.01	0.81		
Val	1.30	1.07*	1.33	1.64	1.41	0.135	0.08	0.79	0.01	0.97	0.99		
Ala	1.12*	1.64	1.41*	0.35	0.31	0.262	0.30	0.31	<0.01	0.23	0.82		
Asn	0.62	0.54	0.61	0.47	0.47	0.044	0.31	0.63	0.07	0.33	0.49		
Asp	0.018	0.016	0.023	0.003	0.028	0.0162	0.47	0.47	0.74	0.40	0.64		
Cys	0.12	0.21	0.11	0.17	0.11	0.064	0.86	0.84	0.76	0.26	0.92		
Gln	1.67	1.72	1.91	1.17*	1.13*	0.147	0.97	0.33	<0.01	0.76	0.48		
Glu	0.49 ^a	0.47 ^a	0.50 ^a	0.23 ^b	0.37 ^{ab}	0.047	0.19	0.65	<0.01	0.08	0.82		
Gly	1.54*	2.68	1.94	1.08*	0.79*	0.427	0.30	0.60	<0.01	0.09	0.66		
Pro	0.24	0.26	0.26	0.13	0.11	0.026	0.99	0.59	<0.01	0.35	0.72		
Ser	0.33	0.47	0.54	0.32	0.35	0.084	0.24	0.04	0.11	0.44	0.48		
Tyr	0.97*	0.93	0.96*	0.76	0.81	0.030	0.74	0.98	<0.01	0.13	0.71		

^aMeans within a row with no common superscripts differ ($P < 0.05$).

¹Abomasal infusion treatments: LMP-C, 0.9% saline; LMP-GG, 1,319 g/d of glucose; LMP-LG, 676 g/d of palm olein; HMP-C, 844 g/d of a complete EAA mixture in the same profile and amount as found in 1.5 kg of casein; HMP-GG, 1,319 g/d of glucose + 844 g/d of EAA; HMP-LG, 676 g/d of palm olein + 844 g/d of EAA. Infusions of LMP-GG, LMP-LG, and HMP-C supplied 20.5 MJ/d, and HMP-GG and HMP-LG supplied 41.0 MJ/d.

²GG = effect of energy from glucose; LG = effect of energy from fat; AA = effect of protein level.

³EAA = Arg (considered semi-essential), His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val.

⁴Group 1 = His, Met, Phe+Tyr, Trp.

⁵Group 2 = Arg, Ile, Leu, Lys, Thr, Val.

⁶BCAA (branched-chain AA) = Ile, Leu, Val.

⁷NEAA = Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, Tyr.

⁸TAA (total AA) = EAA + NEAA.

⁹TAA on an N basis.

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

†Value does not differ from 1.00 (tendency; $0.10 \geq P > 0.05$).

Table 7. Calculated mammary gland glucose and long-chain fatty acid balance in lactating dairy cows (n = 6) receiving abomasal infusions of saline (control; C), glucose (GG), and palm olein (LG) at low (LMP) and high (HMP) metabolizable protein levels for 5 d

Item	Treatment ¹						SEM	P-value ²				
	LMP-C	LMP-GG	LMP-LG	HMP-C	HMP-GG	HMP-LG		GG	LG	AA	GG × AA	LG × AA
Glucose												
Uptake, g/h	66	67	67	50	74	53	7.8	0.07	0.68	0.64	0.14	0.99
Lactose output:glucose uptake	0.80	0.79	0.80	1.26	0.88	1.16	0.095	0.03	0.54	0.03	0.32	0.81
Required for lactose, ³ g/h	54	52	52	60	62	61	3.5	0.96	0.77	<0.01	0.35	0.53
Required for fat, ³ g/h	15	13	17	17	17	19	1.0	0.10	0.09	<0.01	0.29	0.76
Excess, ⁴ g/h	-3.4*	1.2*	-1.9*	-27.7	-4.5*	-26.9	6.10	0.03	0.78	0.02	0.18	0.88
LCFA uptake, ⁵ g/h	15	12	24	14	13	27	3.0	0.42	<0.01	0.29	0.56	0.36
LCFA output, ⁶ g/h	23	18	29	24	19	30	1.3	<0.01	<0.01	0.24	0.84	0.96
LCFA balance, g/h	-8.5	-6.7	-5.8	-10.5	-5.8	-3.7*	2.95	0.17	0.04	0.52	0.54	0.36

¹Abomasal infusion treatments: LMP-C, 0.9% saline; LMP-GG, 1.319 g/d of glucose; LMP-LG, 676 g/d of palm olein; HMP-C, 844 g/d of a complete EAA mixture in the same profile and amount as found in 1.5 kg of casein; HMP-GG, 1.319 g/d of glucose + 844 g/d of EAA; HMP-LG, 676 g/d of palm olein + 844 g/d of EAA. Infusions of LMP-GG, LMP-LG, and HMP-C supplied 20.5 MJ/d, and HMP-GG and HMP-LG supplied 41.0 MJ/d.

²GG = effect of energy from glucose; LG = effect of energy from fat; AA = effect of protein level.

³Requirements for lactose and fat estimated based on calculations of Dijkstra et al. (1996).

⁴Excess = Uptake – required for lactose – required for fat.

⁵Molecular weight of fatty acids (FA) ≥16C in blood was calculated according to the molecular weight of ≥16C FA in milk (Nichols et al., 2019b), assuming 50% of milk C16 originated from C16 FA in blood sequestered as preformed FA and that 50% of milk C16 was synthesized de novo. LCFA = long-chain fatty acids.

⁶Preformed milk FA (>16C) and 50% of milk C16 FA (Nichols et al., 2019b).

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

other individual group 1 AA at the HMP level (His, 1.11; Met, 1.03; Trp, 1.00). At the LMP level, average Phe U:O was 1.05 and in line with previous ranges observed from cows fed low (11.8%) and high (16.5%) CP contents (Crompton et al., 2014). The individual U:O of Tyr decreased from 0.95 on average at the LMP level to 0.78 on average at the HMP level, the latter of which is low compared with the range reported by Crompton et al. (2014). A possible explanation is that the high level of EAA infusion in the current study caused a relative shortage of Tyr and the intramammary hydroxylation of Phe to Tyr increased at the HMP level. If the excess uptake relative to milk output of Phe was converted to Tyr, according to our assumption for the Fick principle, approximately 22% of milk Tyr would have come from mammary-sequestered Phe. Verbeke et al. (1972) suggested that hydroxylation of Phe to Tyr is influenced by Phe concentration, and Jorgensen and Larson (1968) showed that this conversion is affected by presence or absence of Tyr in mammary cells. Both observations would support a high level of conversion of Phe to Tyr when mammary gland Phe uptake increased 54% at the HMP level relative to the LMP level. However, the extent to which intramammary Phe contributes to Tyr synthesis is still not well established, and significant amounts of intracellular Tyr may originate from mammary tissue breakdown or may arise not only from plasma free AA but also from mammary uptake of peptides (Lemosquet et al., 2010; Crompton et al., 2014).

The HMP level decreased net U:O of total AA (TAA). Our EAA infusion rate of 844 g/d is high relative to others infusing EAA mixtures in a casein profile (Doepel and Lapierre, 2010; Maxin et al., 2013; Doelman et al., 2015). When exogenous EAA supply is high and imbalanced relative to NEAA for casein synthesis and intramammary metabolism increases, net U:O might not accurately represent TAA transfer. Although total EAA were extracted by the gland in excess of their requirement, mammary uptake of NEAA was reduced at the HMP level relative to their increased output, resulting in a net U:O of TAA of <1. When calculated on an N basis, the U:O of TAA is closer to unity, which supports contribution of N from group 2 AA to N required for de novo NEAA synthesis (Lapierre et al., 2012). At infusion doses similar to ours, but when casein or mixtures of EAA+NEAA are infused, U:O of TAA are ≥1 (Guinard and Rulquin, 1994; Raggio et al., 2006b; Doepel and Lapierre, 2010). Under the condition of high EAA supply that is also imbalanced relative to NEAA required for casein synthesis, peptide-bound AA may be extracted by the gland to compensate for the deficiency. Evidence of this contribution at low or high protein supplies is equivocal but has been suggested in

scenarios in which AA supply for milk protein synthesis is lacking (Backwell et al., 1994; Bequette et al., 1999, 2001).

Glucogenic Infusion Reduced Group 2 AA Concentration but Milk Protein Yield Was Maintained

Mammary plasma flow increased 22% in response to 1.3 kg/d of infused glucose, which agrees with increases in MPF of 24 and 36% in cows infused for 14 d with 1 kg/d duodenal glucose or ruminal propionate (Rulquin et al., 2004; Raggio et al., 2006b). Mammary plasma flow will increase or decrease to maintain intramammary energy balance via 2C compounds (acetate and BHB; Cant et al., 2003). Glucogenic infusions typically reduce circulating acetate and BHB concentrations (Rigout et al., 2002; Lemosquet et al., 2009; Curtis et al., 2018), which likely stimulates the concomitantly observed hyperaemia. Arterial BHB concentration decreased 36% with glucose infusion in the current study, which is in line with this proposed regulation of MPF and in agreement with Curtis et al. (2018) in which arterial concentration of acetate + BHB decreased 31% and MPF increased 36% in response to 96-h intravenous glucose infusion. Elevated glucose and insulin concentrations observed in response to GG may have reduced arterial BHB through stimulatory effects on lipogenesis in adipose (Vernon et al., 1985), antiketogenic effects in the liver, and BHB oxidation by peripheral tissues (Ørskov et al., 1999).

Arterial plasma concentrations of all group 2 AA, with the exception of Thr, decreased in response to GG, independent of MP level. Others have reported reduced concentrations of group 2 AA upon postruminal infusion of propionate, starch, and glucose (Raggio et al., 2006a; Rius et al., 2010; Nichols et al., 2016), often with no concomitant increase in their net mammary uptake (Raggio et al., 2006b; Nichols et al., 2016; Curtis et al., 2018). In the current study, a GG $\times$ AA interaction affected mammary uptake of Arg, Lys, Ile (tendency), and Leu (tendency) and tended to affect mammary uptake of total group 2 AA, indicating lower uptake of these AA at the LMP level and no effect at the HMP level. Considering that glucose infusion reduced circulating group 2 AA, increased MPF, and did not decrease milk protein yield, it follows that efficiency of group 2 AA uptake increased in response to GG. This increase was detected in the mammary clearance parameter for Ile and Lys but was more significant at the LMP level than at the HMP level for Arg, Leu, Val, and total group 2 AA (GG $\times$ AA interactions). Group 2 AA are typically extracted by the udder in a greater amount relative to their output in milk, and they are used for de novo synthesis of NEAA and as a supply

of glycolytic and tricarboxylic acid cycle intermediates (Mephram, 1987; Lapierre et al., 2012). Validity of inclusion of Thr in this group is equivocal (Lapierre et al., 2012) because its contribution to NEAA and oxidation in the mammary gland may be small (Verbeke et al., 1972). Indeed, its individual U:O in the current study did not differ from 1 on any treatment. Nevertheless, when considered together, total group 2 AA U:O was >1 on all treatments except LMP-GG. Milk protein yield did not decrease on LMP-GG relative to LMP-C or LMP-LG; therefore, during glucose infusion at the LMP level, intramammary compensation must have occurred to yield AA-N and carbon required to synthesize NEAA and generate energy. Of the group 2 AA, individual U:O of Arg remained >1 and thus could still contribute AA-N and carbon. The U:O of NEAA was numerically highest on LMP-GG. Of the NEAA, U:O of Ala and Gly increased most (numerically) on LMP-GG relative to LMP-C and LMP-LG, possibly to offset lower de novo synthesis of these AA. The U:O of Ala appears to be particularly responsive under conditions of low protein intake (Raggio et al., 2006b; Doepel and Lapierre, 2010; Haque et al., 2015) and during insulin infusion (Bequette et al., 2001). In addition, mammary extraction of circulating peptides may contribute relatively more to milk protein synthesis on LMP-GG (Backwell et al., 1994; Bequette et al., 1999; Lapierre et al., 2012), but they were not quantified in the present AV measurements.

Glucose infusion directed a portion of circulating group 2 AA toward utilization in nonmammary tissues, as evidenced by their lower arterial concentration at low and high MP levels, while mammary uptake of group 2 AA was not increased. Metabolism of BCAA and Lys in ruminants occurs predominantly in nonhepatic tissues (Lapierre et al., 2002), and nonmammary sinks for group 2 AA are oxidative catabolism, skeletal muscle protein synthesis, and adipose TAG synthesis (Brosnan and Brosnan, 2006; Raggio et al., 2006a). Partitioning of group 2 AA toward extramammary peripheral tissues in response to GG agrees with the numerical reduction in urinary N output and increase in body N retention reported by Nichols et al. (2019b). Furthermore, decreased plasma urea concentration in response to GG, in particular at the HMP level, is in accordance with overall reduced oxidative catabolism of AA. It also agrees with the observations of Raggio et al. (2006a) showing that whole-body oxidation of Leu was not affected during 14-d propionate infusion and was reduced when propionate and casein were infused together. Arterial concentrations of 1 methyl-histidine and 3 methyl-histidine were not affected by GG, indicating that muscle protein degradation was not increased, which is in line with the antiproteolytic

effect of insulin on muscle (Lobley, 1998). However, GG increased the arterial concentration of Gly, which is released from skeletal muscle at high levels relative to other AA when body protein is mobilized (Doepel et al., 2002). These indicators suggest that turnover in the skeletal muscle pool during glucose infusion could have sequestered some of the group 2 AA. Considering the BCAA specifically, recent work suggests a role for adipose tissue in BCAA metabolism in dairy cattle when glucose increases insulin concentration and stimulates lipogenesis (Nichols et al., 2016; Curtis et al., 2018). Taken together, the increase in insulin and decrease in BHB and NEFA concentrations, the reduction in milk fat yield, and the increase in body energy retention (observed by Nichols et al., 2019b) in response to GG suggest contribution to lipogenesis in adipose was a likely sink for BCAA carbon skeletons during glucose infusion.

In summary, regardless of MP level, glucose infusion decreased the arterial concentration of group 2 AA. At the LMP level, glucose infusion resulted in numerically lower mammary net uptake of group 2 AA, numerically reduced intramammary group 2 AA catabolism (lower U:O), and did not affect milk protein yield. During glucose infusion at the HMP level, mammary net uptake of group 2 AA was not affected and milk protein yield was not affected, but intramammary catabolism of group 2 AA numerically increased (higher U:O). Anabolic effects of insulin on peripheral tissues in response to GG likely accounted for the reduced arterial concentrations of BHB and group 2 AA and the reduced intramammary catabolism of group 2 AA to maintain milk protein synthesis at the LMP level.

Lipogenic Infusion Did Not Affect Mammary AA Utilization

Infusion of palm olein did not affect MPF or mammary net uptake of any AA group. This outcome agrees with the findings of Nichols et al. (2019a) in response to 28-d dietary supplementation with rumen-inert saturated fat. However, those authors did report a tendency for milk protein yield to increase 45 g/d with fat supplementation, whereas in the current study milk protein yield was not affected by LG. Interestingly, Nichols et al. (2019a) report Ser as the only AA to be affected by fat feeding at the level of mammary uptake and clearance, which was also observed in the current study. Data characterizing mammary gland kinetics in response to fat supplementation are limited. Previous work suggested that feeding LCFA might improve mammary AA extraction efficiency, but this improvement could be counteracted by depressed MPF if intramammary energy requirements are reduced when short-chain FA

and glucose are spared by reduced de novo FA synthesis (reviewed by DePeters and Cant, 1992). However, subsequent stimulation of lactose synthesis from spared glucose increases ATP utilization (Cant et al., 2003). According to the hypothesis that MPF is regulated to maintain intramammary energy balance, it follows that palm olein infusion did not affect MPF in the current study because it decreased de novo milk FA synthesis (Nichols et al., 2019b), did not decrease milk lactose yield, and did not affect arterial BHB concentration.

Insulin concentration was not affected by palm olein infusion, and thus the anabolic effects of insulin on peripheral tissues did not initiate partitioning of EAA toward extramammary tissues in response to LG, in contrast with the effect of glucose infusion and in line with our hypothesis. Therefore, a greater portion of circulating AA were available for mammary AA uptake during palm olein infusion, but greater AA uptake was not observed, and milk protein output was not affected. Plasma urea concentration tended to increase with LG at the LMP level and tended to decrease at the HMP level, but it was numerically higher on HMP-LG than on LMP-LG, which is consistent with greater AA catabolism as protein supply increases. In agreement with our hypothesis, although both energy substrates resulted in the same level of milk protein synthesis, glucose infusion resulted in a larger response at the mammary gland level compared with palm olein to sequester AA.

Mammary Glucose and LCFA Balance

The 192 g/d increase in lactose yield stimulated by the HMP level did not coincide with increased mammary glucose uptake in response to EAA infusion. Furthermore, at the HMP level (except when glucose was infused), mammary glucose uptake was insufficient to cover estimated requirements for lactose and fat synthesis, assuming 1.05 and 0.31 g of glucose is required to synthesize 1 g of lactose and fat, respectively (Dijkstra et al., 1996). Reported estimates indicate that 100% of glucose in lactose but only 60 to 80% of galactose in lactose is derived from plasma glucose (Sunehag et al., 2002; Bequette et al., 2006). Amino acids can contribute to carbon substrates required for galactose synthesis by the gland, and this contribution could be affected by absorptive EAA level (Bequette et al., 2006; Maxin et al., 2013). Maxin et al. (2013) observed lower contribution of labeled glucose in milk galactose during abomasal AA infusion, indicating that a larger portion of nonglucose carbon (up to 50%) was used for galactose synthesis in the mammary gland when AA were infused. Bequette et al. (2006) estimated that 12% of galactose synthesized by mammary cells in vitro was derived from EAA catabolism. In the current study,

the U:O of TAA was the furthest from unity at the HMP level. If a portion of AA extracted by the gland contributed to galactose synthesis, this would not be accounted for in our TAA balance or in our glucose balance but would reduce the apparent glucose deficit for lactose synthesis. In response to abomasal infusion of the same EAA profile and dose, the reported lactose yield expressed relative to reported mammary glucose uptake by Nichols et al. (2016) was 1.12, comparable with the values of 1.26 and 1.16 on HMP-C and HMP-LG in the current study.

Palm olein infusion did not affect circulating glucose or net mammary glucose uptake but produced the same level of lactose yield as glucose infusion, independent of MP level. Absorption of LCFA into circulation promotes their direct incorporation into milk fat, thereby reducing de novo FA synthesis and glucose requirements for oxidative catabolism, which in turn may spare some intramammary glucose for lactose synthesis (Chilliard, 1993; Hammon et al., 2008). Mammary net uptake of TAG and LCFA ($\geq 16C$) increased in response to LG, and milk LCFA ($\geq 16C$) output increased (Nichols et al., 2019b). However, LCFA uptake equilibrated with LCFA output in milk only with HMP-LG infusion. A larger portion than the assumed 50% of C16 may have been synthesized de novo on the other treatments, which would mitigate some or all of the calculated shortfall in net mammary supply of LCFA from blood relative to milk LCFA. However, with the assumption of more 16C FA being synthesized de novo in the gland, intramammary glucose requirement would become increasingly deficient to support glycerol synthesis and yield NADPH. The concentration of FA $< 16C$, assumed to be 100% synthesized de novo, decreased in milk fat with palm olein infusion (Nichols et al., 2019b), which would allow some glucose sparing. Increased mammary uptake of BHB at the HMP level could have resulted in more than 50% of 16C FA synthesized de novo, which would partly offset the apparent deficit in LCFA balance at the HMP level.

CONCLUSIONS

Increased absorptive supply of glucose and palm olein differentially affected mammary gland metabolite utilization, irrespective of MP level. Anabolic effects of insulin on extramammary peripheral tissues during glucose infusion likely accounted for the reduced arterial concentrations of BHB and group 2 AA and increased MPF, regardless of MP level, and the reduced intramammary catabolism of group 2 AA at the LMP level. We suspect glucose stimulated lipogenesis in adipose and increased MPF to maintain intramammary ATP balance. Regardless of protein level, palm olein did not

promote an insulin response and did not affect arterial AA concentrations or mammary AA utilization. The HMP level increased milk protein yield, increased uptake of all EAA groups, and increased intramammary catabolism of group 2 AA and BCAA, but it decreased that of NEAA. At the HMP level, mammary glucose uptake was insufficient to cover estimated requirements for lactose and fat synthesis, except when glucose was infused. This deficit may have been mitigated through shifts in intramammary metabolite partitioning, such as contribution of EAA carbon to galactose, and decreased de novo 16C FA synthesis. These results suggest that lactose secretion is not solely dependent on mammary glucose supply at high MP levels, and they illustrate the flexibility of mammary metabolite utilization.

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