Maintenance of plasma branched-chain amino acid concentrations during glucose infusion directs essential amino acids to extra-mammary tissues in lactating dairy cows

Richelle V. Curtis,* Julie J. M. Kim,* John Doelman,† and John P. Cant*¹

*Department of Animal Biosciences, University of Guelph, ON, N1G 2W1 Canada

†Trouw Nutrition Agresearch Canada, Guelph, ON, N1G 4T2 Canada

ABSTRACT

The objectives of this study were to investigate the effects of branched-chain AA (BCAA) supplementation when glucose is infused postruminally into lactating dairy cows consuming a diet low in crude protein (CP) and to test the hypothesis that low BCAA concentrations are responsible for the poor stimulation of milk protein yield by glucose. Twelve early-lactation Holstein cows were randomly assigned to 15% and 12% CP diets in a switchback design of 6-wk periods. Cows consuming the 12% CP diet received 96-h continuous jugular infusions of saline and 1 kg/d of glucose with 0, 75, or 150 g/d of BCAA in a Latin square sequence of treatments. Compared with saline, glucose infusion did not affect dry matter intake but increased milk yield by 2.2 kg/d and milk protein and lactose yields by 63 and 151 g/d, respectively. Mammary plasma flow increased 36% during glucose infusion compared with saline infusion, possibly because of a 31% decrease in total acetate plus β -hydroxybutyrate concentrations. Circulating concentrations of total essential AA and BCAA decreased 19 and 31%, respectively, during infusion of glucose, yet net mammary uptakes of AA remained unchanged compared with saline infusion. The addition of 75 and 150 g/d of BCAA to glucose infusions increased arterial concentrations of BCAA to 106 and 149%, respectively, of the concentrations in saline-infused cows, but caused a decrease in concentrations of non-branched-chain essential AA in plasma, as well as their mammary uptakes and milk protein yields. Plasma urea concentration was not affected by BCAA infusion, indicating no change in catabolism of AA. The lack of mammary and catabolic effects leads us to suggest that BCAA exerted their effects on plasma

concentrations of the other essential AA by stimulating utilization in skeletal muscle for protein accretion. Results indicate that the glucose effect on milk protein yield was not limited by low BCAA concentrations, and that a stimulation of extra-mammary use of non-branched-chain essential amino acids by BCAA led to a decrease in milk protein yield.

Key words: mammary uptake, branched-chain amino acid, glucose, milk synthesis

INTRODUCTION

Of the total digestible EAA that make it into portal blood from the gastrointestinal tract of the lactating dairy cow, approximately 50% is taken up by the mammary glands and used for milk protein production (Lapierre et al., 2012). The remaining half enters into body protein or is catabolized in the liver to produce glucose and ATP. Provision to the cow of additional glucose, precursors of glucose such as propionate, or euglycemic insulin spares NEAA from entering into gluconeogenesis (Lemosquet et al., 2004; Curtis et al., 2014) and directs EAA into protein of both the muscle and the mammary glands (Clark et al., 1977; Bequette et al., 2002; Rulquin et al., 2004). However, evidence of glucose or glucose precursors effectively stimulating milk protein yield has been inconsistent, as some studies show a positive effect (Rulquin et al., 2004; Raggio et al., 2006a) and other studies do not (Hurtaud et al., 1998; Curtis et al., 2014; Nichols et al., 2016).

Concentrations in plasma of the branched-chain amino acids (BCAA) Ile, Leu, and Val decline when glucose or propionate is supplied (Raggio et al., 2006a; Curtis et al., 2014; Nichols et al., 2016), which may contribute to small or absent milk protein yield responses to glucose. Nichols et al. (2016) estimated that approximately 136 g/d of BCAA were diverted into non-mammary tissues as a result of infusing 1 kg/d glucose. When 215 g/d of BCAA was subtracted from 1,475 g/d of MP, milk protein yield decreased 123 g/d (Doelman et al., 2015b), suggesting that a loss of 136

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¹Corresponding author: jcant@uoguelph.ca

g/d of BCAA is sufficiently large to interfere with the ability of glucose to stimulate milk protein production.

The present experiment was designed to investigate the effects of BCAA replacement when glucose is infused postruminally into lactating dairy cows consuming a low-CP diet and to test the hypothesis that low BCAA concentrations are responsible for the poor stimulation of milk protein yield by glucose. Circulating concentrations and net mammary uptakes of energy metabolites and AA were measured during infusions of 75 and 150 g/d of BCAA to quantify the supply of postruminal BCAA needed to counteract the decrease in plasma BCAA concentrations elicited by 1 kg/d glucose, and to gather evidence as to which pathways of BCAA utilization are responsible for the loss.

MATERIALS AND METHODS

Animals, Diets, and Feeding Management

All experimental procedures were approved by the Animal Care Committee at the University of Guelph (Guelph, ON, Canada). Twelve Holstein cows in their second lactation began the experiment at 80 ± 22 d of lactation and weighed 700 ± 44.3 kg. Cows were housed in tie stalls, given individual free access to water and feed throughout the study, and milked twice daily at 0500 and 1700 h. Two TMR were formulated at 15% (normal) and 12% (low) CP to meet 105 and 87% of MP requirements and 105 and 101% of ME requirements, respectively, for 36 kg/d of milk (NRC, 2001; Table 1). The mean balances for MP, RDP, and RUP for the normal- and low-CP diets, respectively, were formulated to be 127.5 and -339 g/d, -32.3 and 265.2 g/d, and 143 and -309.7 g/d. Additionally, mean balances for EAA, Met, and Lys for the normal- and low-CP diets, respectively, were formulated at 76 and -143.6 g/d, 0.75 and -10.3 g/d, and 1.2 and 29.5 g/d. The 15% CP (normal) ration represented a characteristic corn alfalfa silage-based diet fed to Ontario dairy cattle for the purpose of establishing normal milk production and protein efficiency values with which to compare responses to glucose and BCAA infusions on the 12% CP (low) TMR. Cows were fed at 1200 h daily, and feed offered and refused was weighed to determine daily ad libitum feed intakes of individual cows. Samples of each ration were collected daily, stored at -20°C , and pooled every other week over the 16-wk study for nutrient composition, which was analyzed by wet chemistry at a local commercial laboratory (Agri-Food Labs, Guelph, ON, Canada). Feed refusal samples were collected daily from individual cows, stored at -20°C , and pooled weekly. Dry matter contents of feed and

refusal samples were determined using a forced-air oven at 55°C for 48 h.

Experimental Design, Catheterizations, and Jugular Infusions

Cows were assigned the normal- and low-CP diets in a switchback design of two 6-wk periods. The first 2 wk were allocated for diet adaptation, and cows assigned to the low-CP diet were subjected to a series of infusions for the remaining 4 wk. Each of these 4 wk consisted of 96 h of continuous infusion, followed by 72 h of washout with no infusion, in a randomized, Latin square sequence of treatments. Cows on the normal-CP diet did not receive infusions.

Infusion treatments on the low-CP diet were 0.9% saline (SAL), 1 kg/d of glucose (GLC), 1 kg/d of GLC + 75 g/d of BCAA (GLC+75), or 1 kg/d of GLC + 150 g/d of BCAA (GLC+150). The BCAA

Table 1. Ingredient composition and nutrient analysis (% of DM unless otherwise noted) of the 2 experimental diets

Component	Low CP	Normal CP
Ingredient		
Haylage	45.4	28.5
Corn silage	21.7	34.7
Corn, high-moisture	26.7	20.5
Straw, long chop	3.6	3.6
Soy plus ¹	0	4.7
Soybean meal	0	3.0
Canola meal	0	1.5
Fish meal	0	0.5
Biuret	0	0.1
Urea	0.2	0.03
Vitamin/mineral mix ²	2.4	2.8
Nutrient		
CP	12.6	14.9
Soluble CP, % of CP	32.8	39.1
NDICP, ³ % of CP	23.0	21.7
ADICP, ⁴ % of CP	10.1	8.4
NDF	35.0	32.8
ADF	21.8	20.4
Lignin	10.5	9.6
NFC	45.9	46.5
Starch, % of NFC	56.6	55.0
Ether extract	3.1	3.1
NE _L , ⁵ Mcal/kg	1.52	1.59

¹West Central Cooperative, Ralston, Iowa.

²Obtained from Floradale Feed Mill (Floradale, ON, Canada) and consisted of calcium (6.6%), phosphorus (5.0%), sodium (12.5%), sulfur (2.7%), magnesium (6.5%), iron (2,900 mg/kg), zinc (2,833 mg/kg), manganese (2,244 mg/kg), fluorine (483 mg/kg), monensin sodium (460 mg/kg), copper (414 mg/kg), iodine (33.7 mg/kg), cobalt (33.3 mg/kg), selenium (12 mg/kg), vitamin A (436 kIU/kg), vitamin D (70.3 kIU/kg), and vitamin E (1,287 IU/kg).

³NDICP = neutral detergent-insoluble CP.

⁴ADICP = acid detergent-insoluble CP.

⁵Calculated according to NRC (2001).

were supplied in a 1:1:1 ratio by weight of Leu:Ile:Val. Treatment solutions were mixed daily by completely dissolving GLC and BCAA in 4 L of water set to pH 12.0 with 10 M NaOH, adjusting pH to 7.4 with HCl, followed by sterilization via 0.2- μ m filtration. Saline was autoclaved for sterilization. Infusion treatments were administered through jugular catheters via peristaltic pump at a rate of approximately 2.78 mL/min. Infusions began and ended on d 1 and 5, respectively, of each infusion sub-period at 1200 h.

Approximately 3 d before the onset of infusions, cows on the low-CP diet were fitted with long-term catheters (14-gauge, 20 cm; MILA International Inc., Erlanger, KY) in a jugular vein. Catheters were checked daily and were replaced in the opposite jugular vein in the case of patency failure. Ceftiofur (1 mg/kg of BW; Zoetis Canada, Kirkland, QC, Canada) was administered subcutaneously for 3 consecutive days during infusion weeks.

Milk, Blood Sampling, and Tissue Biopsies

During infusion sub-periods, milk samples were collected at 0500 and 1700 h on the last 3 d of infusion. Samples were stored at 4°C, and submitted within 24 h for fat, protein, and lactose analysis by infrared spectroscopy (AOAC International, 1996; Laboratory Services Division, University of Guelph, Guelph, ON, Canada). Blood samples were collected according to Nichols et al. (2016). Briefly, 4 sets of blood samples were taken simultaneously every 2 h by needle puncture from the subcutaneous mammary abdominal vein and a coccygeal vessel into potassium EDTA and sodium heparin Vacutainers (Becton Dickinson, Rutherford, NJ) on d 4 of each infusion sub-period, commencing at 0800 h. Collection tubes were immediately placed on ice and centrifuged at $3,000 \times g$ for 15 min to separate blood plasma. Plasma was aliquoted into polypropylene tubes to be stored at -20°C for further analysis. Longissimus dorsi, subcutaneous adipose, and mammary samples were collected on the last day of each 96-h infusion sub-period following the morning milking for a companion study. Milk, blood, and tissue samples were collected from cows on the normal-CP diet after 4 wk on the diet, equivalent in time to infusion sub-period 2.

Plasma Analysis

Plasma samples for each cow were pooled over time by sub-period and analyzed for glucose, insulin, acetate, BHB, nonesterified fatty acids (NEFA), and triacylglycerol (TAG), as described by Weekes et al. (2006); free glycerol was analyzed according to Bucolo

and David (1973). Urea nitrogen was analyzed according to Sampson et al. (1980) and insulin was analyzed by ELISA (Crystal Chem Inc., Downers Grove, IL). Plasma AA concentrations for each time point were analyzed using ultra performance liquid chromatography in conjunction with Empower Chromatography Data software (Waters Corp., Milford, MA), according to the protocol described by Boogers et al. (2008).

Calculations and Statistical Analysis

Energy balance of cows was estimated as $NE_{\text{INTAKE}} - NE_{\text{MILK}} - NE_{\text{MAINT}}$, where NE_{INTAKE} was calculated from observed DMI and its estimated net energy content. Net energy supplies from GLC, GLC+75, and GLC+150 infusates were estimated to be 2.75, 3.05, and 3.36 Mcal/d, respectively, based on 2.75 Mcal/kg of glucose (Hurtaud et al., 1998) and 4.06 Mcal/kg of BCAA, equal to 64% of their summed heats of combustion. Net energy of milk (NE_{MILK}) was obtained from the regression equation of Tyrrell and Reid (1965) against observed milk fat, protein, and lactose yields, and $NE_{\text{MAINT}} = 0.08BW^{0.75}$ (NRC, 2001).

Mammary plasma flow (MPF) was predicted in accordance with the Fick principle, using Phe and Tyr as internal markers (Cant et al., 1993), where MPF (L/h) = milk Phe + Tyr output (μ mol/h)/arteriovenous Phe + Tyr difference (μ mol/L). Using Phe and Tyr contents reported by Lapierre et al. (2012), Phe + Tyr output in milk was predicted from $0.965 \times$ milk protein yield during the blood-sampling interval. Mammary uptakes of individual metabolites were calculated as the arteriovenous concentration difference (AVD) $\times$ MPF. Net removal from plasma by the mammary gland is indicated by a positive uptake, whereas net release from the mammary gland is indicated by a negative uptake. Hydrolysis of TAG in the capillary lumen releases NEFA that are available for mammary uptake in addition to NEFA from the arterial inflow. Total arterial long-chain fatty acid concentrations were calculated as $3 \times \text{TAG AVD} + \text{arterial [NEFA]}$. Mammary clearance of metabolites was calculated as uptake/venous concentration (Cant et al., 2016).

Dry matter intake, milk yield, and milk composition were averaged over the last 2 d of each infusion sub-period. Plasma AA concentrations were averaged over the 4 sampling times on d 4 of each infusion sub-period. Variances in cow performance, plasma concentrations, mammary uptakes, and clearances were analyzed as an incomplete randomized block design, with cow, period, and sub-period as blocking factors. The 4 infusion treatments on low-CP diets were distributed evenly across all 4 sub-periods, whereas measurements on the

Table 2. Performance of lactating dairy cows ($n = 12$) consuming a normal (15%) CP diet or a low (12%) CP diet with i.v. glucose and branched-chain AA (BCAA) for 96 h¹

Item	Treatment ²					SEM	P-value
	Normal CP	Low CP					
		SAL	GLC	GLC+75	GLC+150		
DMI, kg/d	25.9 ^a	20.8 ^b	19.8 ^{bc}	19.4 ^c	18.9 ^c	0.66	<0.01
NE _L intake, Mcal/d	39.4 ^a	31.7 ^b	32.8 ^b	32.6 ^b	32.1 ^b	1.00	<0.01
Yield							
Milk kg/d	31.9 ^a	26.3 ^c	28.5 ^b	27.0 ^{bc}	26.2 ^c	2.09	<0.01*
Fat, g/d	1,142	1,131	1,172	1,124	1,070	68.0	0.39*
Protein, g/d	1,030 ^a	767 ^c	830 ^b	756 ^c	731 ^c	47.3	<0.01*
Lactose, g/d	1,492 ^a	1,230 ^c	1,381 ^b	1,310 ^{bc}	1,266 ^c	103.7	<0.01*
Composition, %							
Fat	3.69 ^a	4.40 ^b	4.15 ^b	4.25 ^b	4.22 ^b	0.210	0.01
Protein	3.33 ^a	2.96 ^b	2.97 ^b	2.86 ^b	2.87 ^b	0.132	<0.01
Lactose	4.70	4.67	4.85	4.82	4.81	0.068	0.15
NE _L balance, Mcal/d	6.7 ^a	1.7 ^b	1.4 ^b	2.3 ^b	2.6 ^b	0.99	<0.01
Protein efficiency, %	30.6	27.9	31.8	29.4	29.5	1.43	0.12

^{a-c}Values within a row with different superscripts differ significantly at $P \leq 0.05$.

¹Data are least squares means from the final 2 d of each sub-period.

²Infusion treatments were 0.9% saline (SAL), 1 kg/d of glucose (GLC), 1 kg/d of glucose + 75 g/d of BCAA (GLC+75), or 1 kg/d of glucose + 150 g/d of BCAA (GLC+150). BCAA (Val, Leu, and Ile) were infused in a 1:1:1 weight ratio.

* $P \leq 0.05$: linear effect of BCAA.

normal-CP treatment were taken during sub-period 2 only, representing the middle of the 6-wk period. The following model was solved using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC):

$$Y_{ijk} = \mu + \text{cow}_i + \text{per}_j + \text{sub}_k + \text{trt}_l + \varepsilon_{ijkl},$$

where μ = overall mean, cow_i = random effect of cow ($i = 1$ to 12), per_j = fixed effect of 6-wk period ($j = 1$ to 2), sub_k = fixed effect of infusion sub-period ($k = 1$ to 4), trt_l = fixed effect of treatment ($l = 1$ to 5), and ε_{ijkl} = experimental error. Due to sampling difficulty from one cow, plasma concentrations, mammary uptakes, and clearances of metabolites and AA had a sample size of 11. Effects were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.15$. Least-squares treatment means were separated using the pdiff option of SAS when overall treatment $P < 0.05$ (SAS Institute Inc.). Effects of BCAA were estimated as pre-planned linear contrasts between GLC, GLC+75, and GLC+150, and were declared significant when P -values for quadratic contrasts were not significant.

RESULTS

DMI and Milk Yield and Composition

Dry matter intake tended to decrease 1.0 kg/d with GLC ($P = 0.07$) compared with SAL (Table 2), but addition of BCAA to GLC had no effect on DMI ($P =$

0.14). Calculated intakes of NE_L did not differ between SAL, GLC, GLC+75, and GLC+150. Infusion of GLC increased milk, protein, and lactose yields by 2.2 kg/d, 63 g/d, and 151 g/d, respectively ($P \leq 0.05$), and had no effect on fat yield ($P = 0.43$) compared with SAL. The addition of BCAA to GLC infusions elicited linear decreases in all component yields and total milk yield ($P \leq 0.05$). Milk concentrations of fat, protein, and lactose, NE_L balance, and protein efficiency were unaffected by infusion treatments, except that GLC increased lactose concentration by 0.18 percentage units ($P = 0.04$) compared with SAL.

Cows on the normal-CP diet consumed an average of 6.1 kg/d more DMI ($P < 0.01$) and 7.3 Mcal/d more NE_L ($P < 0.01$) than cows on the low-CP diet and produced 4.9 kg/d more milk ($P < 0.01$). On average, NE_L balance was 4.9 Mcal/d more positive ($P < 0.01$) for cows on the normal-CP diet. Daily milk protein yield was, on average, 259 g higher ($P < 0.01$) and lactose yield was 195 g/d higher ($P < 0.05$) when cows were fed the normal-CP ration. Protein percentage was elevated ($P < 0.01$) and fat percentage reduced ($P < 0.02$) in milk of cows fed the normal-CP diet compared with those on the low-CP diet. Fat yield and protein efficiency were unaffected by dietary CP content.

Arterial Insulin and Metabolite Concentrations

Infusion treatments on the low-CP diet did not affect plasma insulin, glucose, glycerol, NEFA, TAG, long-

chain fatty acids, or urea concentrations compared with SAL ($P \geq 0.18$; Table 3). Although infusion of GLC did not significantly affect acetate or BHB concentrations, total 2C (acetate + $2 \times$ BHB) concentration decreased with GLC ($P = 0.01$) and GLC+150 ($P = 0.01$) infusions, and GLC+75 tended ($P = 0.07$) to decrease plasma 2C concentration compared with SAL.

Infusion of GLC decreased ($P \leq 0.016$) plasma concentrations of the EAA Arg, Ile, Leu, Lys, and Val so that total circulating EAA and BCAA concentrations

decreased 19 and 31%, respectively ($P \leq 0.01$), compared with SAL (Table 3). Non-branched-chain EAA (NBEAA) concentrations were not different between GLC and SAL ($P = 0.16$). Of the individual NEAA, plasma concentrations of Orn decreased ($P < 0.04$) and Ser increased ($P = 0.01$) with GLC infusion. Total AA concentrations were not different between GLC and SAL ($P = 0.62$).

We detected no effect of BCAA on insulin, energy metabolites, or urea concentrations in plasma. Infusion

Table 3. Arterial plasma concentrations of metabolites and insulin in lactating dairy cows ($n = 11$) consuming a normal (15%) CP diet or a low (12%) CP diet with i.v. glucose and branched-chain AA (BCAA) for 96 h¹

Item	Treatment ²					SEM	P-value
	Normal CP	Low CP					
		SAL	GLC	GLC+75	GLC+150		
Insulin, ng/mL	4.1	3.6	3.5	3.5	4.1	1.04	0.91
Glucose, mM	3.15	3.06	3.02	3.19	3.30	0.181	0.53
Acetate, mM	1.28	1.33	1.06	1.12	1.04	0.132	0.25
BHB, mM	0.95 ^a	0.55 ^b	0.39 ^{bc}	0.47 ^{bc}	0.40 ^c	0.077	<0.01
2C, ³ mM	3.17 ^a	2.47 ^b	1.70 ^c	2.02 ^{bc}	1.83 ^c	0.207	<0.01
Glycerol, μM	13.0	21.5	18.4	20.4	22.2	2.68	0.29
Nonesterified fatty acids, μM	109.4	73.8	88.6	81.6	69.8	18.39	0.74
Triacylglycerol, μM	31.6 ^a	17.8 ^b	21.2 ^b	19.7 ^b	19.8 ^b	2.54	0.04
Long-chain fatty acids, ⁴ μM	193	133	152	145	125	21.1	0.35
Urea, mM	1.17 ^a	0.61 ^b	0.61 ^b	0.62 ^b	0.62 ^b	0.059	<0.01
Amino acids, ⁵ μM							
TAA	2,434	2,302	2,263	2,425	2,394	83.3	0.15†
EAA	824 ^a	695 ^c	566 ^d	679 ^c	777 ^b	41.9	<0.01*
BCAA	443 ^a	336 ^b	232 ^c	357 ^b	501 ^a	27.8	<0.01*
NBEAA	381 ^a	359 ^a	334 ^{ab}	322 ^b	276 ^c	19.8	<0.01*
NEAA	1,610	1,606	1,698	1,746	1,617	60.8	0.06
Arg	86 ^a	61 ^b	51 ^c	51 ^c	46 ^c	4.5	<0.01†
His	27 ^a	16 ^b	14 ^b	13 ^b	12 ^b	2.2	<0.01
Ile	113 ^a	85 ^b	57 ^c	84 ^b	110 ^a	7.4	<0.01*
Leu	101 ^a	83 ^b	59 ^c	85 ^b	117 ^a	5.8	<0.01*
Lys	80 ^a	60 ^b	48 ^c	47 ^c	41 ^c	4.4	<0.01†
Met	22 ^a	19 ^b	17 ^b	16 ^b	13 ^c	1.3	<0.01*
Phe	37 ^{ab}	38 ^a	35 ^{ab}	34 ^b	33 ^b	1.9	0.03†
Thr	87 ^a	129 ^b	133 ^b	126 ^b	99 ^a	11.5	<0.01*
Trp	42 ^a	36 ^{bc}	36 ^b	35 ^{bc}	33 ^c	1.6	0.02*
Val	230 ^b	168 ^c	116 ^d	188 ^c	274 ^a	15.2	<0.01*
Ala	261	257	234	246	240	15.2	0.24
Asn	52 ^a	41 ^{bc}	43 ^{bc}	44 ^b	40 ^c	2.1	0.02
Gln	423	386	426	452	402	22.2	0.10
Glu	133	118	110	121	101	11.5	0.38
Gly	401	496	562	555	518	38.0	0.06
Orn	83 ^a	53 ^b	44 ^c	43 ^c	38 ^c	5.3	<0.01
Pro	100 ^a	75 ^b	71 ^b	71 ^b	64 ^c	3.9	<0.01*
Ser	109 ^a	139 ^a	166 ^b	176 ^b	179 ^b	12.8	<0.01
Tyr	48 ^a	41 ^{bc}	43 ^{ab}	37 ^{cd}	32 ^d	2.7	<0.01*

^{a-d}Values within a row with different superscripts differ ($P \leq 0.05$).

¹Data presented as least squares means from d 4 of each sub-period.

²Infusion treatments were 0.9% saline (SAL), 1 kg/d of glucose (GLC), 1 kg/d of glucose + 75 g/d of BCAA (GLC+75), or 1 kg/d of glucose + 150 g/d of BCAA (GLC+150). BCAA (Val, Leu, and Ile) were infused in a 1:1:1 weight ratio.

³2C = acetate + $2 \times$ BHB.

⁴Arterial LCFA = $3 \times$ triacylglycerol arteriovenous difference + arterial nonesterified fatty acids.

⁵TAA = total amino acids (EAA + NEAA); NEAA = Asn, Ser, Gln, Gly, Glu, Ala, Pro, Orn, and Tyr; EAA = His, Arg, Thr, Lys, Met, Val, Ile, Leu, Phe, and Trp; BCAA = Val, Ile, and Leu; NBEAA (non-branched-chain EAA) = EAA – BCAA.

* $P \leq 0.05$, † $P \leq 0.10$: linear effect of BCAA.

of BCAA caused a linear increase in plasma concentrations of Ile, Leu, and Val ($P < 0.01$) to levels that were not different from SAL at 75 g/d of BCAA ($P > 0.1$) and to levels higher than SAL at 150 g/d of BCAA ($P < 0.01$; Table 3). The NBEAA Met, Thr, and Trp declined linearly in concentration ($P < 0.05$) as BCAA infusion rate increased, and Arg, Lys, and Phe concentrations tended to decline ($P \leq 0.15$), so that total NBEAA concentrations decreased ($P = 0.002$). The increases in concentrations of BCAA were greater than the decreases in NBEAA, resulting in linear increases in total EAA concentrations ($P \leq 0.01$) due to BCAA infusion. Of the NEAA, Pro and Tyr decreased linearly ($P \leq 0.05$) with BCAA infusion but total NEAA was not affected ($P = 0.13$). Total AA concentration in plasma tended to increase linearly with BCAA infusion rate ($P = 0.08$).

Arterial concentrations of BHB, 2C, TAG, and urea were higher on the normal-CP diet than on the low-CP diet ($P < 0.03$). Dietary CP level did not affect insulin, glucose, acetate, glycerol, or NEFA concentrations. An elevation in plasma concentrations of all EAA except Phe, Thr, and Trp was observed in cows on normal- versus low-CP diets. Of the NEAA, only Asn, Orn, Pro, and Tyr concentrations increased, and total NEAA concentration was not affected by dietary CP level. Total AA concentration was not higher on the normal-CP diet.

Mammary Plasma Flow and Metabolite Uptakes

Mammary plasma flow increased 36% with GLC infusion compared with SAL ($P < 0.01$) but net mammary uptakes of energy metabolites were not affected ($P > 0.10$; Table 4). Mammary net uptakes of amino acids, both individually and as groups, remained similar between GLC and SAL infusions ($P > 0.24$; Table 4). A linear increase due to BCAA infusion was observed in net mammary uptakes of Val, Ile, and Leu ($P < 0.03$) whereas net uptakes of Arg, Lys, Met, Phe, Ala, and total NBEAA linearly decreased due to BCAA infusion ($P \leq 0.05$).

Mammary plasma flow and mammary net uptakes of energy metabolites remained similar between dietary CP levels. Mammary net uptakes of all EAA except His and Trp, and the NEAA Asn, Orn, and Ser, were higher on the normal-CP diet than on the low-CP diet ($P < 0.05$), resulting in elevated net uptakes of all groups of EAA and total AA (Table 4).

Infusions did not affect rate constants for net clearance of energy metabolites from plasma by the mammary glands ($P > 0.10$). However, GLC infusion stimulated an increase in net mammary clearance of EAA (P

$= 0.04$), which was due entirely to an increase in BCAA clearance ($P < 0.01$). None of the NBEAA clearances were affected by GLC. Similarly, addition of BCAA to GLC infusions did not affect NBEAA clearances except for a tendency for Arg clearance to decline ($P = 0.09$). Infusing BCAA linearly decreased clearances of Val, Ile, Leu, and Ala ($P \leq 0.01$), linearly increased Tyr clearance ($P = 0.02$), and tended to increase Glu clearance ($P = 0.08$).

Mammary clearances of energy metabolites remained similar between dietary CP levels (Table 5). Higher clearances of Thr, Asn, and Ser ($P \leq 0.05$) were observed in cows on the normal-CP diet than on the low-CP diet. Total AA clearance was elevated on the higher CP diet ($P < 0.02$).

DISCUSSION

Glucose Infusion Decreased Plasma BCAA Concentrations

The main objective of our study was to determine whether small or negligible milk protein yield responses to glucose are due to the depletion of BCAA from plasma. A low-CP ration was chosen as the base diet to intensify effects on milk protein yield of infusion-induced changes in circulating AA concentrations. The i.v. infusion of GLC at 1 kg/d induced a modest 63 g/d, or 8% over basal, increase in milk protein yield, despite a decrease in exogenous MP and EAA supply due to 1 kg/d lower DMI, and a 31% decrease in concentration of BCAA in plasma. These results indicate that GLC improved the efficiency of capture of MP in milk protein. Previously, postruminal glucose or propionate supplementation stimulated milk protein yield in some studies (Rulquin et al., 2004; Raggio et al., 2006a; Al-Trad et al., 2009) and had no effect in others (Hurtaud et al., 1998; Curtis et al., 2014; Nichols et al., 2016). In all experiments, however, efficiency of capture of MP in milk protein was improved by glucose.

Net mammary uptakes of AA were not affected by GLC, which suggests that instead of diverting dietary AA to the mammary glands, as has been observed in previous studies (Rulquin et al., 2004; Raggio et al., 2006b), GLC caused AA taken up by the mammary glands to be partitioned more into milk protein and less into catabolism. Euglycemic insulin infusion has a similar effect, where milk protein synthesis is stimulated despite a reduction in net AA uptakes by the mammary glands (Mackle et al., 2000; Bequette et al., 2001).

Uptakes of BCAA, Lys, and Arg did not decrease even though their arterial concentrations decreased. The improvement in efficiency of uptake of BCAA from

Table 4. Mammary plasma flows and net uptakes of metabolites in lactating dairy cows ($n = 11$) consuming a normal (15%) CP diet or a low (12%) CP diet with i.v. glucose and branched-chain AA (BCAA) for 96 h¹

Item	Treatment ²					SEM	P-value
	Low CP						
	Normal CP	SAL	GLC	GLC+75	GLC+150		
Mammary plasma flow, L/h	1,007 ^{ab}	828 ^a	1,127 ^{bc}	1,077 ^b	1,275 ^c	95.2	<0.01
Net mammary uptake, mmol/h							
Glucose	669	424	644	532	870	190.0	0.42
Acetate	520	614	774	778	810	122.4	0.52
BHB	270 ^a	90 ^b	132 ^b	180 ^a	184 ^a	35.6	0.02
2C ³	1,081	883	1,174	1,247	1,266	150.0	0.10
Glycerol	−2.1	4.3	4.1	7.7	10.6	3.84	0.21†
Nonesterified fatty acids	−1.6	−5.3	8.0	1.5	−11.3	9.22	0.49†
Triacylglycerol	19.8	10.6	15.3	15.6	15.3	2.47	0.16
Long-chain fatty acids	61.7	30.0	58.1	52.0	38.4	11.23	0.19
Amino acids ⁴							
TAA	480 ^a	300 ^b	321 ^b	288 ^b	305 ^b	43.0	0.12
EAA	253 ^a	168 ^b	170 ^b	169 ^b	182 ^b	17.2	0.03
BCAA	124 ^a	80 ^b	79 ^b	84 ^b	104 ^a	9.2	<0.01*
NBEAA	129 ^a	88 ^b	90 ^b	85 ^{bc}	78 ^c	8.4	<0.01*
NEAA	227	132	152	119	123	31.9	0.33
Arg	27.9 ^a	16.5 ^{bc}	17.3 ^b	16.6 ^{bc}	14.2 ^c	1.86	<0.01*
His	10.8	7.4	7.2	6.7	7.0	0.89	0.07
Ile	32.4 ^a	20.6 ^b	19.5 ^b	22.2 ^b	27.6 ^a	2.41	<0.01*
Leu	47.0 ^a	31.5 ^c	31.1 ^c	31.5 ^c	36.4 ^b	3.35	<0.01*
Lys	40.5 ^a	30.0 ^b	29.2 ^b	27.1 ^{bc}	25.4 ^c	2.69	<0.01*
Met	8.9 ^a	6.6 ^{bc}	6.7 ^b	6.1 ^{bc}	5.5 ^c	0.73	0.04*
Phe	16.1 ^a	11.5 ^{bc}	12.2 ^b	11.4 ^{bc}	10.5 ^c	1.13	<0.01*
Thr	21.6	13.7	15.1	14.1	12.5	2.15	0.14
Trp	3.3	2.0	2.6	2.7	3.1	0.50	0.39
Val	44.3 ^a	28.4 ^b	28.6 ^b	30.1 ^b	39.9 ^a	4.00	<0.01*
Ala	34.4	21.9	24.2	13.7	11.5	5.83	0.07*
Asn	10.2 ^a	4.2 ^b	5.0 ^b	3.6 ^b	3.7 ^b	1.08	<0.01
Gln	58.2	39.0	39.6	41.0	37.2	9.66	0.77
Glu	17.8	21.0	27.3	9.3	17.9	9.48	0.59
Gly	27.2	4.9	13.1	18.5	16.6	16.99	0.92
Orn	31.8 ^a	22.5 ^b	21.3 ^b	20.3 ^b	20.4 ^b	2.20	0.02
Pro	9.9	6.8	7.8	6.7	6.4	1.71	0.76
Ser	22.7 ^a	1.9 ^b	2.7 ^b	−4.0 ^b	−0.9 ^b	4.74	0.03
Tyr	15.0	10.0	10.7	9.9	10.0	1.15	0.06

^{a-c}Values within a row with different superscripts differ significantly at $P \leq 0.05$.

¹Data presented as least squares means from d 4 of each sub-period.

²Infusion treatments were 0.9% saline (SAL), 1 kg/d of glucose (GLC), 1 kg/d of glucose + 75 g/d of BCAA (GLC+75), or 1 kg/d of glucose + 150 g/d of BCAA (GLC+150). BCAA (Val, Leu, and Ile) were infused in a 1:1:1 weight ratio.

³2C = acetate + 2 × BHB.

⁴TAA = total amino acids (EAA + NEAA); NEAA = Asn, Ser, Gln, Gly, Glu, Ala, Pro, Orn, and Tyr; EAA = His, Arg, Thr, Lys, Met, Val, Ile, Leu, Phe, and Trp; BCAA = Val, Ile, and Leu; NBEAA (non-branched-chain EAA) = EAA - BCAA.

* $P \leq 0.05$, † $P \leq 0.10$: linear effect of BCAA.

extracellular fluid was large enough to be detected in the clearance parameters. Mammary plasma flow and clearance are positively associated (Madsen et al., 2015) so the 36% increase in MPF during GLC infusion may have contributed to these higher clearances. Increases in MPF of 24 and 36% were previously observed in cows infused for 14 d with 1 kg/d of duodenal glucose or ruminal propionate (Rulquin et al., 2004; Raggio et al., 2006b). Euglycemic insulin infusion also increases MPF (Mackle et al., 2000; Bequette et al., 2001). These hyperemias can be attributed to glucose- or insulin-

induced decreases in circulating 2C concentrations observed in this and other studies (Eisemann and Huntington, 1994; Rigout et al., 2002; Lemosquet et al., 2009) if it is assumed, according to Cieslar et al. (2014), that (1) net mammary uptakes of energy metabolites are dependent on their respective arterial concentrations and MPF, (2) intramammary ATP production is dependent on energy metabolite uptakes, and (3) vasodilator release into the mammary interstitium is a function of intracellular ATP balance. Combined, these hypotheses predict that MPF will increase to maintain

Table 5. Mammary clearances (L/h) of metabolites in lactating dairy cows ($n = 11$) consuming a normal (15%) CP diet or a low (12%) CP diet with i.v. glucose and branched-chain AA (BCAA) for 96 h¹

Metabolite	Treatment ²					SEM	P-value
	Normal CP	Low CP					
		SAL	GLC	GLC+75	GLC+150		
Glucose	305	166	277	214	395	103.2	0.43
Acetate	2,041	1,477	2,233	1,996	2,259	803.5	0.94
BHB	855	108	700	647	1,132	373.9	0.28
2C ³	636	835	1,388	1,170	1,227	216.7	0.09
Triacylglycerol	2,148	4,459	3,715	4,125	3,264	2173.4	0.96
Long-chain fatty acids	576	304	592	543	404	110.0	0.16
Amino acids ⁴							
TAA	252 ^a	155 ^b	165 ^b	132 ^b	144 ^b	24.4	0.07
EAA	508 ^{ab}	348 ^{bc}	443 ^a	329 ^{bc}	281 ^c	51.4	<0.01*
BCAA	460 ^b	378 ^b	603 ^a	320 ^b	232 ^b	76.2	<0.01*
NBEAA	563	349	372	362	388	43.9	0.07
NEAA	163	92	102	73	83	22.6	0.22
Arg	570	439	605	542	494	100.2	0.17†
His	1,073	1,498	1,626	1,752	2,056	386.6	0.41
Ile	492 ^b	389 ^b	681 ^a	401 ^b	300 ^b	88.1	<0.01*
Leu	1,115 ^b	784 ^b	1,241 ^a	580 ^{bc}	346 ^c	157.6	<0.01*
Lys	1,259	1,366	1,614	1,442	1,444	187.7	0.58
Met	724	694	831	701	785	117.3	0.84
Phe	868	528	555	530	491	80.0	0.10
Thr	331 ^a	120 ^b	123 ^b	121 ^b	144 ^b	32.6	<0.01
Trp	91	60	76	84	198	14.8	0.30
Val	284 ^b	239 ^b	378 ^a	200 ^b	159 ^b	57.4	0.01*
Ala	165 ^a	100 ^{ab}	129 ^a	57 ^b	37 ^b	32.3	0.03*
Asn	252 ^a	120 ^b	133 ^b	91 ^b	108 ^b	29.8	0.03
Gln	181	122	101	108	120	31.2	0.63
Glu	209	200	367	172	155	98.4	0.40†
Gly	60	9	31	38	36	33.1	0.88
Orn	765	960	1,013	962	1,061	138.7	0.68
Pro	115	108	132	114	120	31.0	0.97
Ser	204 ^a	23 ^b	33 ^b	−15 ^b	0 ^b	32.9	<0.01
Tyr	482	372	321	366	428	48.8	0.10*

^{a-c}Values within a row with different superscripts differ significantly at $P \leq 0.05$.

¹Data presented as least squares means from d 4 of each sub-period.

²Infusion treatments were 0.9% saline (SAL), 1 kg/d of glucose (GLC), 1 kg/d of glucose + 75 g/d of BCAA (GLC+75), or 1 kg/d of glucose + 150 g/d of BCAA (GLC+150). BCAA (Val, Leu, and Ile) were infused in a 1:1:1 weight ratio.

³2C = acetate + 2 × BHB.

⁴TAA = total amino acids (EAA + NEAA); NEAA = Asn, Ser, Gln, Gly, Glu, Ala, Pro, Orn, and Tyr; EAA = His, Arg, Thr, Lys, Met, Val, Ile, Leu, Phe, and Trp; BCAA = Val, Ile, and Leu; NBEAA (non-branched-chain EAA) = EAA - BCAA.

* $P \leq 0.05$, † $P \leq 0.10$: linear effect of BCAA.

intramammary energy balance when the total concentration of energy metabolites in plasma decreases or vice versa (Cant et al., 2003). The decrease in plasma 2C concentration during glucose or insulin infusion is due to stimulation of lipogenesis and FA esterification in adipose tissue (Vernon et al., 1985), anti-ketogenic effects in the liver, and BHB utilization in non-hepatic tissues (Ørskov et al., 1999).

Although the increase in MPF may have prevented a decrease in uptakes of BCAA, Lys, and Arg during GLC infusion, it did not cause an increase in net mammary uptakes or clearances of the other EAA. If additional NBEAA were transported into mammary cells because of the faster MPF, they apparently were not

used and must have returned to the venous circulation without contributing to net uptake. This disregard of available NBEAA suggests that milk protein synthesis was under the control of other factors, possibly the BCAA supply. Branched-chain AA and Leu subtraction studies have shown a reduction in milk protein yield when plasma concentrations of Leu decreased to 42 or 48 μM and total BCAA concentrations decreased to 245 μM (Rulquin and Pisulewski, 2006; Doelman et al., 2015b), whereas no milk protein depression was reported when plasma Leu and BCAA concentrations decreased to 63 and 297 μM , respectively (Weekes et al., 2006). Plasma concentrations of Leu and BCAA of 59 and 232 μM , respectively, in the present study were

at or below milk protein-depressing levels and may have limited the GLC-induced increase in milk protein yield.

The decline in plasma BCAA concentrations when glucose is delivered postruminally to dairy cattle is well documented in the literature (Hurtaud et al., 1998; Raggio et al., 2006a; Rius et al., 2010; Nichols et al., 2016). The absence of an increase in mammary BCAA uptakes during glucose infusions in this and other studies (Raggio et al., 2006b; Nichols et al., 2016) indicates that glucose directs BCAA to non-mammary tissues. Possible sinks for the BCAA are skeletal muscle protein, oxidative catabolism, and adipose TAG. Skeletal muscle protein contains 2.2, 5.7, and 3.1 g/100 g of Ile, Leu, and Val, respectively, but it is richer in Lys, Thr, and Phe, which account for 9.1, 4.5, and 4.3 g/100 g, respectively (Early et al., 1990). Considering, in addition, that portal appearances of BCAA are greater than or equal to appearances of Lys, Thr, and Phe (Lapierre et al., 2006), stimulation of muscle protein deposition by glucose should not affect plasma concentrations of BCAA more than it affects concentrations of Lys, Thr, and Phe. Concentrations of BCAA were affected to a much greater degree than Lys, Thr, and Phe, so muscle protein deposition does not seem to account for their specific loss. Nichols et al. (2016) found no effect on expression in muscle of genes involved in BCAA catabolism and suggested that glucose stimulates BCAA utilization for lipogenesis in adipose tissue and thereby decreases plasma BCAA concentrations in the same manner that it causes a decrease in 2C and NEFA concentrations.

When 75 and 150 g/d BCAA were added to glucose infusions, plasma BCAA concentrations returned to 106 and 149%, respectively, of the concentrations in SAL cows. A linear interpolation indicates that 60 g/d BCAA would have been necessary to maintain plasma BCAA levels when 1 kg/d of glucose was infused. Nichols et al. (2016) estimated the utilization of BCAA induced by 1 kg/d of glucose on a high plane of EAA nutrition to be 136 g/d, using a linear relationship between rate of BCAA infusion and plasma BCAA concentration. The difference between estimates may be related to plasma BCAA concentrations on the control without glucose, which averaged 1,500 μ M in the study by Nichols et al. (2016) and 336 μ M in the current study.

BCAA Infusion Decreased Plasma NBEAA Concentrations

If loss of BCAA into extra-mammary tissues during glucose infusion was preventing glucose from stimulating milk protein yield, then yield should have been higher on GLC+75 than on GLC, when BCAA concentrations

were returned to SAL levels by infusion. Milk protein yield actually decreased when BCAA were replaced, and decreased even further at GLC+150, as evidenced by a linear effect of BCAA infusion. When BCAA concentrations in plasma increased due to BCAA infusion, net BCAA uptake by the mammary glands increased but net mammary BCAA clearance decreased, indicating that not all of the available BCAA were used. Additional BCAA taken up by the mammary glands must have been catabolized because sequestration in milk protein was reduced. This catabolism may have provided N for Ala synthesis, the net uptake of which decreased linearly with BCAA infusion, and also likely yielded ATP for milk synthesis.

Infusion of BCAA caused a 17% decline in NBEAA concentrations in plasma. In particular, Lys, Met, and Thr concentrations decreased 15, 24, and 26%, respectively, in cows receiving 150 versus 0 g/d of BCAA. Although DMI was not significantly affected by BCAA infusion, mean DMI was 5% lower on GLC+150 than on GLC, which may have contributed to some of the reduction in NBEAA concentrations. The remainder of the decline was not due to faster or more efficient mammary uptakes and outputs of NBEAA in milk protein. Effects of BCAA infusion on plasma urea concentrations were not detected in this study, suggesting that catabolism was also not contributing to a reduction in plasma concentrations of NBEAA. The lack of mammary and catabolic effects leads us to suggest that BCAA exerted their effects on plasma NBEAA by stimulating utilization in skeletal muscle for protein accretion.

There is much supporting evidence for a stimulatory effect of BCAA on muscle protein deposition. Leucine is a potent activator of the mechanistic (mammalian) target of rapamycin complex 1 (**mTORC1**) pathway that upregulates protein synthesis in skeletal muscle of growing animals (Escobar et al., 2006). In lactating cows, 5 d of abomasal infusion of EAA increased mTORC1 target phosphorylation in skeletal muscle (Nichols et al., 2017). This mTORC1 effect may be the mechanism by which i.v. infusion of a complete mix of AA stimulated muscle protein synthesis in lactating goats (Bequette et al., 2002). A stimulatory effect of BCAA on muscle protein deposition explains the decrease in plasma concentrations of Lys, Arg, Thr, and Met observed by Rulquin and Pisulewski (2006) in cows receiving duodenal Leu infusions during which milk protein yield was not affected, and the decrease in milk urea concentrations observed in cows abomasally infused with BCAA when milk protein yield was also not affected (Appuhamy et al., 2011). When BCAA were omitted from a complete EAA mix infused into the abomasum of lactating cows, milk protein yield

did not decrease and it was suggested that loss of the anabolic signal from BCAA in muscle diverted AA from muscle into milk (Weekes et al., 2006). Combined, these responses give precedent to the suggestion that NBEAA concentrations decreased because of a stimulatory effect of 75 and 150 g/d BCAA on muscle protein deposition.

The 17% decrease in plasma NBEAA concentrations during BCAA infusion appears to have been responsible for the observed 12% depression in milk protein yield. Studies in which single EAA subtractions from post-ruminal infusates negatively affect milk protein yield have shown that His is deficient at a plasma concentration of 13 μ M, Lys is deficient at 56 μ M, Met at 10 μ M, and Phe at 25 μ M (Weekes et al., 2006; Lapierre et al., 2009; Doelman et al., 2015a,b; Doepel et al., 2016). The concentration of Lys in cows on the GLC treatment in our experiment was below its deficient value, at 48 μ M, and concentrations of His, Met, and Phe were not much above, at 14, 17, and 35 μ M, respectively. The significantly decreased concentrations of Lys and Met to lower-than-deficient levels during BCAA infusion suggests that milk protein yield was depressed by reduced arterial concentrations of these AA.

It is interesting that BCAA infusion appeared to stimulate muscle protein synthesis but did not stimulate milk protein synthesis, despite evidence that a portion of the additional BCAA were taken up by the mammary glands and therefore available to stimulate milk protein synthesis. Both Leu and Ile are stimulatory to mTORC1 and protein synthesis in mammary cells in vitro (Moshel et al., 2006; Appuhamy et al., 2012; Arriola Apelo et al., 2014). Correction of a BCAA deficiency in lactating cows increased mammary abundances of mRNA translation regulators (Doelman et al., 2015b), which is thought to be a consequence of mTORC1 activation in vivo. The diversion of NBEAA away from milk protein and into muscle suggests that protein synthesis is more sensitive to BCAA concentrations in muscle than in the mammary glands of lactating cows. Using labeled Leu kinetics to estimate protein synthesis rates, Bequette et al. (2002) noted a similar difference between skeletal muscle and mammary glands in sensitivity to intravenous infusion of a complete AA mixture in goats, where the anabolic effect was stronger in muscle.

Glucose Plus BCAA Did Not Overcome NBEAA Shortfalls

A normal-CP diet was included in the present study to establish normal milk production and mammary uptake values with which to compare low-CP diet ob-

servations. Cows on the normal-CP diet had 263 g/d higher milk protein yields than cows on the low-CP SAL treatment. As expected, supplying more dietary CP produced higher plasma concentrations and net mammary uptakes of EAA. Infusion of GLC on the low-CP diet stimulated milk protein yield by a modest 63 g/d but not through an increase in mammary uptake of EAA or NEAA. Addition of BCAA to GLC infusions caused a decrease in mammary uptake of NBEAA that paralleled their loss out of circulation to extra-mammary tissues. Mammary BCAA uptakes on the low-CP GLC+150 treatment were not different from those on the high-CP diet but NBEAA and NEAA uptakes and milk protein yield remained 40%, 46%, and 299 g/d lower, respectively. The combination of 1 kg/d glucose and 150 g/d BCAA was stimulatory to extra-mammary utilization of NBEAA to the detriment of milk protein production.

CONCLUSIONS

In the present study, we demonstrated that infusion of 1 kg/d of glucose into the jugular vein of lactating dairy cows consuming a diet of 12% CP increased milk protein yield by 63 g/d and reduced plasma BCAA concentrations by 31%. Returning BCAA concentrations to the level observed without glucose infusion did not further stimulate milk protein yield, indicating that the glucose effect was not limited by low BCAA concentrations. Interpolation of the response in plasma BCAA concentrations to infusions of 0, 75, and 150 g/d of BCAA yielded an estimate of 60 g/d for the glucose-stimulated BCAA loss. Branched-chain AA were not taken up by the mammary glands to a greater extent during glucose infusion, and stimulation of muscle protein deposition would be expected to influence Lys, Thr, and Phe concentrations to a greater extent than BCAA concentrations, which was not observed. It is possible that BCAA concentrations were reduced by the same mechanism that reduced acetate + BHB concentrations. Supplementing glucose infusions with BCAA triggered a decrease in milk protein yield that appeared to be a consequence of lower concentrations of NBEAA in circulation. Constant plasma urea concentrations across infusion treatments suggest that NBEAA did not disappear by catabolism. It is likely that BCAA infusions directed NBEAA into the protein of skeletal muscle rather than that of milk.

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