Abomasal amino acid infusion in postpartum dairy cows: Effect on whole-body, splanchnic, and mammary amino acid metabolism

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ABSTRACT

Nine Holstein cows with rumen cannulas and indwelling catheters in splanchnic blood vessels were used in a generalized randomized incomplete block design with repeated measures to study the effect of increased early postpartum AA supply on splanchnic and mammary AA metabolism. At calving, cows were blocked according to parity (second and third or greater) and allocated to 2 treatments: abomasal infusion of water (CTRL; $n = 4$) or free AA with casein profile (AA-CN; $n = 5$) in addition to a basal diet. The AA-CN infusion started with half of the maximal dose at the calving day (1 d in milk; DIM) and then steadily decreased from 791 to 226 g/d until 29 DIM. On 5, 15, and 29 DIM, 6 sample sets of arterial, portal, hepatic, and mammary blood were taken at 45-min intervals. Over the whole period, increasing AA supply increased milk ($+7.8 \pm 1.3$ kg/d) and milk protein yields ($+220 \pm 65$ g/d) substantially. The increased milk yield was not supported by greater dry matter intake (DMI) as, overall, DMI decreased with AA-CN (-1.6 ± 0.6 kg/d). Arterial concentrations of essential AA were greater for AA-CN compared with CTRL. The net portal-drained viscera (PDV) release of His, Met, and Phe was greater for AA-CN compared with CTRL, and the net PDV recovery of these infused AA ranged from 72 to 102% once changes in DMI were accounted for. The hepatic removal of these AA was increased equivalently to the increased net PDV release, resulting in an unaltered net splanchnic release. The net PDV release of Ile, Leu, Val, and Lys tended to be greater for AA-CN, and the net PDV recovery of these infused AA ranged from 69 to 73%, indicating increased PDV metabolism with AA-CN. The

fractional hepatic removal of these AA did not differ from zero and was unaffected by the increased supply. Consequently, the splanchnic release of these AA was approximately equivalent to their net PDV release for both CTRL and AA-CN. Overall, greater early postpartum AA supply increased milk and milk protein yields substantially based on increased mammary AA uptake. The PDV metabolism of branched-chain AA and Lys were increased, whereas it seemed to be unaffected for other essential AA when the intestinal AA supply was increased. On a net basis, the liver removed more group 1 AA (His, Met, Phe, and Trp) for anabolism and catabolism when the early postpartum AA supply was increased. Thus, increasing the postpartum AA supply increased splanchnic and mammary consumption of AA; hence, the protein deficiency persisted.

Key words: dairy cow, parturition, amino acid, splanchnic, mammary

INTRODUCTION

It has long been recognized that the postpartum cow is in negative energy and protein balance because the high demand created by initiation of lactation is not covered by equivalent increases in nutrient supply from dietary intake. The consequences of negative energy balance and associated fat mobilization have received great focus during the past decades (e.g., Grummer, 1995; Ingvarsen et al., 2003; Looor, 2010). In contrast, the concomitant negative protein balance has received little attention, even though the extent and potential negative consequences of negative protein balance have been acknowledged (Grummer, 1995; Bell et al., 2000). The extent of postpartum negative protein balance was estimated to be greatest around 7 d postpartum, diminishing thereafter and becoming positive during wk 4 postpartum (Bell et al., 2000). Recently, a similar pattern was observed by relating the release of EAA from total splanchnic tissues to the secretion of EAA into milk protein (Larsen and Kristensen, 2009; Dalbach et al., 2011). Consequently, AA are mobilized during this period from skeletal muscles (Gibb et al., 1992) and

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postpartum involution of smooth muscle in the uterus (Gibb et al., 1992; Andrew et al., 1994).

Several decades ago, Ørskov et al. (1977) observed a positive response in milk production to casein infusion in early lactation cows compared with no response to glucose infusion. Similarly, Larsen et al. (2014b) observed a large increase in milk and milk protein yields in response to an abomasal casein infusion initiated at calving. In this later study, approximately two-thirds of the additional protein supply was utilized to support the increased milk protein synthesis. However, the partitioning of the additionally supplied AA in the early postpartum period among splanchnic, mammary, and other peripheral tissues has yet to be investigated. Available quantitative data on hepatic uptake of AA in the periparturient dairy cows have indicated that the fractional hepatic removal of individual EAA is greater prepartum and in the first week postpartum compared with later in the postpartum period, despite a lower AA supply (Doepel et al., 2009; Larsen and Kristensen, 2013). However, no data are available on the effect of increasing AA supply in the first weeks of lactation on the coordination of AA metabolism between portal-drained viscera (PDV), hepatic tissue, and mammary gland.

Therefore, the overall objective of the present study was to investigate the effect of increasing the AA supply to postpartum dairy cows on splanchnic and mammary AA metabolism, and on whole-body AA balance. We hypothesized that abomasal infusion of AA will increase the net PDV release of AA and, despite a concomitant increase in hepatic AA removal, the net splanchnic release of AA will increase, thereby allowing increased mammary AA uptake and milk protein yield.

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Committee for Animal Care at Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre in Sherbrooke (QC, Canada), and the animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009).

Animals and Experimental Design

Nine multiparous Holstein cows (Table 1) were used in a generalized randomized incomplete block design with repeated measurements (5, 15, and 29 DIM). Cows were blocked according to parity (second: $n = 4$, >second: $n = 5$; Table 1) and, within block, randomly assigned to 1 of 2 treatments: continuous abomasal infusion of either water (CTRL, $n = 4$) or a mixture

Table 1. Description of cows, calf birth weights (mean $\pm$ SD), and treated illnesses

Item	Treatment ¹	
	CTRL	AA-CN
Number of cows	4	5
Second parity, no.	2	2
>Second parity, no.	2	3
BW at dry off, kg	735 $\pm$ 94	735 $\pm$ 104
Previous 305-d ECM, kg	10,410 $\pm$ 1,561	10,294 $\pm$ 1,361
Calf birth weight, kg	40.0 $\pm$ 5.0	44.9 $\pm$ 6.2
Treated illnesses, no.		
Calving problems	—	1
Mastitis	—	1
Metritis	—	—
Milk fever	—	1
Leg problems	—	—
Digestion disorders	—	1
Other ²	1	3

¹Treatments were continuous abomasal infusion of water (CTRL) or a mixture of free AA with casein profile (AA-CN) from 1 to 29 DIM.

²Breath sounds, udder cleft dermatitis, foot fungus.

of free AA, all L-isomers, with casein profile (AA-CN; $n = 5$), both initiated on day of calving (designated as 1 DIM).

Before calving (11–20 wk), cows were fitted with rumen cannulas (Bar Diamond Inc., Parma, ID) according to Duffield (1999). During the dry period, approximately 7 wk before planned calving, cows were surgically implanted with a catheter in the hepatic portal vein, hepatic vein, 2 mesenteric veins, and 1 mesenteric artery (Huntington et al., 1989). During the surgery, the right carotid artery was also raised to a subcutaneous position to provide access to arterial blood if necessary. On the day of calving, an abomasal infusion device was placed in the abomasum by hand through the ruminal cannula and the reticular-omasal orifice. The infusion device was constructed with a flange to secure abomasal placement (Gressley et al., 2006) and was checked manually every third day and in the morning of each sampling day.

The AA-CN rate of infusion was (mean $\pm$ SD) 791 $\pm$ 12 g/d from 2 to 6 DIM, followed by daily decreases of 28.3 $\pm$ 1.0 g/d, with 3-d plateaus around the blood sampling days, giving 558 $\pm$ 2 g/d at 14 to 16 DIM and 226 $\pm$ 0.5 g/d at 28 to 30 DIM. At 1 DIM, the AA-CN rate of infusion was half of the maximal dose. The rates of infusion were estimated to cover the estimated decreasing EAA deficit with advancing DIM (Dalbach et al., 2011). The AA mixture was composed according to the AA composition of casein except that Tyr was replaced by Phe and part of the Glu by Gln, due to solubility constraints, as described previously (Galindo et al., 2011). The AA mixture was prepared every second or third day by mixing in hot tap water. Each day,

8,050 g/d of either water or AA mixture was infused for both treatments using peristaltic pumps; the decreasing AA infusion rate with the AA-CN treatment was obtained by diluting the AA mixture with tap water.

All cows received the same mixed diet prepartum and the same mixed diet postpartum containing 142 and 164 g of CP/kg of DM, respectively, as described in detail in the companion paper (Galindo et al., 2015). The postpartum diet was balanced to provide 100% of NE_L and MP requirements for an estimated intake of 25 kg/d and milk production of 40 kg/d, 3.85% fat, and 3.20% CP (NRC, 2001), the expected DMI and milk yield to be reached by these cows at their plateau. Postpartum, the mixed diet was fed ad libitum (10% orts) and was offered in equal meals every even hour from automated feeders (Ankom Technology, Fairport, NY). In addition to the mixed diets, cows were offered hay ad libitum prepartum through to 2 DIM, 2 kg/d (as-is) from 3 to 6 DIM, and 1 kg/d (as-is) thereafter. Orts were removed at 0900 h and cows were milked at 0800 and 2000 h. Cows had free access to water and were housed in tiestalls bedded with mattresses and wood shavings. To prevent hypocalcemia in cows of third and greater parity, 100 mL of Theracalcium (Vetoquinol N.-A. Inc., Lavaltrie, QC, Canada) was injected i.m. at the first signs of calving, immediately after calving, and once between 12 and 24 h after calving.

Experimental Samplings

Sampling days were scheduled to be 5, 15, and 29 DIM (mean $\pm$ SD: 5.3 ± 0.8 , 15.7 ± 1.2 , and 28.8 ± 1.2 DIM), when splanchnic and mammary blood was sampled. Feed intake and milk yield were determined daily, and diets and ingredients were sampled weekly. Daily milk yield was recorded using continuous flow sampling; however, milk yield was recorded by weighing for the 3 d around sampling days where milk samples were obtained at each milking. Milk samples were stored at -20°C until analysis.

On each sampling day, 6 sets of arterial, portal venous, and hepatic venous blood samples were collected simultaneously at 45-min intervals beginning at 1000 h. Mammary venous blood samples were taken by venipuncture immediately after withdrawal of the splanchnic blood samples. To determine splanchnic plasma flows, continuous infusion of *para*-aminohippuric acid (pAH; 12 g/h; Sigma-Aldrich, St. Louis, MO) into one mesenteric vein using a syringe pump was initiated 30 min before the first sample. Blood was stabilized in Na-heparinized tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) and immediately put on ice; plasma was harvested by centrifugation at $1,800 \times g$ for 12 min at 4°C . Immediately after, 1 g of plasma

was weighed and mixed with 0.2 g of internal standard of labeled AA containing stable isotopes (Doepel and Lapierre, 2010), and stored at -80°C until analysis of AA concentrations. Arterial plasma was subsampled for urea-N analysis. The remaining plasma was stored at -20°C for further analyses.

Analytical Procedures

The DM content of diets and feeds was determined by forced-air oven drying at 55°C for 24 h (48 h for silage). Each milk sample was analyzed for concentrations of total N. The fractions of NPN, casein-N, and whey protein-N in milk protein were obtained as described previously (Raggio et al., 2004) in milk samples pooled within cow and sampling day proportionately to each milking. All milk N determinations were obtained using a Kjeltec auto sampler system (Kjeltec 2400, Foss Analytical AB, Höganäs, Sweden).

All analyses on blood and plasma were conducted on individual samples. Hematocrit was determined immediately after sampling in heparin-stabilized arterial samples by microcentrifugation in capillary tubes at room temperature (20°C). Plasma concentrations of pAH were determined after deacetylation of pAH by heating the samples at 90°C for 2 h in acidic conditions (Lobley et al., 1995). Heparinized plasma samples were analyzed for AA by GC-MS using the isotope dilution method (Calder et al., 1999) with (heptafluorobutyric) AA derivative for Arg, and the *N*-(*tert*-butyldimethyl) AA derivative for all other AA (Doepel and Lapierre, 2010). Arterial plasma concentrations of urea-N were determined in fresh plasma samples at the sampling day using a Technicon AutoAnalyzer system (Seal Analytical Inc., Mequon, WI; Huntington, 1984). Plasma total protein was determined using a biuret reaction end-point assay (ABX Pentra Total Protein CP, Horiba ABX, Montpellier, France) and albumin was determined using a bromocresol green assay (ABX Pentra Albumin CP, Horiba ABX); both assays were adapted for use on a Cobas Mira autoanalyzer (Triolab A/S, Brøndby, Denmark).

Calculations and Statistical Procedures

Milk CP content was calculated by multiplying milk total N content by 6.38 (Sjaunja et al., 1991). True protein and casein yields were calculated by multiplying the ratio between the respective N fraction and total N by the CP yield. The MP balance was estimated by difference between total MP supply [MP supply from the diet estimated using NRC (2001) plus infused AA assumed 100% absorbed] and MP requirement calculated for cow $\times$ day of sampling observation using NRC

(2001). The marginal utilization of infused AA for milk protein with AA-CN was calculated as the ratio between the increment in milk true protein yield and estimated increment in MP supply, as described below, for each cow $\times$ day of sampling observation. Whole-blood concentrations of urea-N were calculated by correcting for the dilution space in erythrocytes of 79.9% (Røjen et al., 2011). Portal venous and hepatic venous plasma flows were calculated by downstream dilution of pAH (Katz and Bergman, 1969). Mammary plasma flow was estimated according to the Fick principle, using Phe and Tyr as internal markers (Cant et al., 1993), allowing for a 3.37% contribution from blood-derived proteins (Lapierre et al., 2012). Concentrations of Phe and Tyr in milk proteins synthesized in the mammary gland were estimated at 51.9 and 58.7 g/kg of true protein, and secretion of AA in milk proteins synthesized by the mammary gland was estimated using the milk AA concentration reported by Lapierre et al. (2012). The net fluxes of metabolites were calculated by multiplying venous-arterial concentration differences with appropriate plasma flows as previously described (Huntington et al., 1996). The mammary uptake to output in milk true protein ratio (U:O) of individual AA was calculated by dividing mammary net flux by secretion in milk true protein using the milk AA concentration of protein synthesized within the mammary gland reported by Lapierre et al. (2012). To allow comparison between treatments while taking into account variation in DMI, the proportion of measured net PDV, net hepatic, and net mammary fluxes having dietary origin in AA-CN was estimated by multiplying the observed DMI by the average net PDV, hepatic, and mammary fluxes per kilogram of DMI for CTRL, respectively. The change in net PDV, hepatic, and mammary fluxes due to abomasal AA infusion with AA-CN were subsequently estimated by subtracting the net fluxes with dietary origin from the measured net PDV, hepatic, and mammary fluxes with AA-CN, respectively. The balance between the amount of AA abomasally infused and the summated increase in PDV, hepatic, and mammary use with AA-CN was then estimated by difference.

Data were subjected to ANOVA using the MIXED procedure in SAS software (version 9.3, TS1M1; SAS Institute Inc., Cary, NC). Sampling day observations were averaged by cow $\times$ DIM before statistical analyses. Daily data (milk and DMI) and sampling day data were both analyzed using a model including fixed effects of block (parity), treatment (Trt), DIM, and the Trt $\times$ DIM interaction. The effect of DIM was considered a repeated measurement using the autoregressive order 1 covariance structure. Denominator degrees of freedom were calculated using the Kenward-Rogers method. Least squares means $\pm$ standard errors of the

mean are presented due to the unbalanced design: one cow assigned to CTRL treatment calved earlier than predicted and could not be used in the experiment. Significance was declared at $P \leq 0.10$ and tendencies were considered at $0.10 < P \leq 0.20$, because of the limited statistical power to test treatment effect in randomized designs (needed in transition cow studies) with limited repetitions (the number of splanchnic surgeries was limited due to technical and ethical constraints). Fisher's least significant difference test (df = 6) was used to separate treatment means within DIM when $P_{\text{Trt} \times \text{DIM}} \leq 0.20$ using the Slice option in SAS. The difference $\pm$ standard error of the difference between overall treatment means was calculated using the Estimate option in SAS. Student's *t*-test was used to determine whether fractional hepatic uptakes differed from zero and whether mammary uptake to output ratios differed ($P \leq 0.10$) from unity.

RESULTS

The single calving problem encountered (Table 1) was solved by light assistance and did not require veterinary

Table 2. Calculated abomasal infusion rates (mmol/h) of AA in a mixture with casein profile (AA-CN) as obtained on different sampling days

Item	DIM		
	5	15	29
EAA	120	86	34
G1 EAA ¹	34	24	9.6
G2 EAA ¹	68	49	19
Arg	6.8	4.9	2.0
His	5.7	4.1	1.6
Ile	12	8.8	3.5
Leu	23	16	6.5
Lys	17	12	4.9
Met	6.3	4.5	1.8
Phe ²	20	14	5.7
Thr	12	8.4	3.4
Trp	1.9	1.4	0.5
Val	16	11	4.5
NEAA	130	93	37
Ala	11	8.0	3.2
Asn	9.3	6.7	2.7
Asp	7.5	5.4	2.1
Cys	1.1	0.8	0.3
Gln ³	36	26	10
Glu ³	9.7	6.9	2.8
Gly	8.0	5.7	2.3
Pro	30	21	8.5
Ser	18	13	5.0
Tyr ²	—	—	—
Total AA	250	180	72

¹Group (G)1 EAA = His, Met, Phe, Tyr, and Trp; G2 EAA = Ile, Leu, Lys, and Val.

²Tyr was substituted with Phe in AA mixture due to low solubility of Tyr.

³Glu was partially replaced by Gln due to low solubility of Glu.

assistance. The other illness were unproblematic and cows responded quickly to veterinary treatment.

Feed Intake and Milk Production

From 1 to 29 DIM, voluntary DMI (including hay) was, on average, 1.6 ± 0.6 kg/d lower ($P_{\text{Trt}} = 0.02$; Figure 1a) for AA-CN compared with CTRL (20.4 ± 0.5 kg/d), with no interaction ($P_{\text{Trt} \times \text{DIM}} = 0.32$). However, considering DMI on sampling days, an interaction was observed ($P_{\text{Trt} \times \text{DIM}} = 0.10$; Table 3) such that DMI was lower at 5 DIM but greater at 29 DIM for AA-CN compared with CTRL. Hay DMI did not differ between treatments ($P_{\text{Trt}} = 0.69$; data not given in tables) and

averaged ($\pm$ SE) 1.5, 0.9, and 0.8 ± 0.1 kg/d at the 5, 15, and 29 DIM sampling days, respectively. The abomasal AA infusion combined with variations in DMI resulted in a constantly higher postpartum total MP supply for AA-CN compared with CTRL ($P_{\text{Trt}} = 0.03$).

From 1 to 29 DIM, milk yield was, on average, 7.8 ± 1.3 kg/d greater ($P_{\text{Trt}} < 0.01$; Figure 1b) for AA-CN relative to CTRL (38.2 ± 1.0) as also observed when considering milk yield on sampling days ($P_{\text{Trt}} = 0.03$; Table 3). Milk true protein yield was greater for AA-CN compared with CTRL ($P_{\text{Trt}} = 0.01$) and decreased as lactation progressed for both treatments ($P_{\text{DIM}} = 0.03$). The average ($\pm$ SD) marginal utilization of infused AA was $55 \pm 4\%$ for milk CP and $48 \pm 5\%$ for milk true protein. The CP content in milk did not differ between treatments ($P_{\text{Trt}} = 0.31$) and decreased as lactation progressed with both treatments ($P_{\text{DIM}} < 0.01$). The composition of milk CP was, however, affected by treatment: the proportion of true protein was 1.1 ± 0.3 percentage units lower ($P_{\text{Trt}} = 0.01$) and the proportion of casein tended to be 3.4 ± 2.4 percentage units lower ($P_{\text{Trt}} = 0.18$) for AA-CN compared with CTRL. The proportions of true protein and casein in milk CP did not differ among DIM ($P_{\text{DIM}} \geq 0.74$). The negative whole-body MP balance did not differ between treatments ($P_{\text{Trt}} = 0.30$) but diminished from 5 to 29 DIM ($P_{\text{DIM}} < 0.01$).

Figure 1. Voluntary DMI (a) and milk yield (b) in postpartum Holstein cows subjected to continuous abomasal infusion of water (○; CTRL; $n = 4$) or a mixture of free AA with casein profile (□; AA-CN; $n = 5$), both initiated on day of calving. On average, voluntary DMI was 1.6 ± 0.6 kg/d lower with AA-CN ($P = 0.02$) compared with CTRL and no interaction between treatment and DIM was detected ($P_{\text{Trt} \times \text{DIM}} = 0.32$). Through the experimental period, milk yield was 7.8 ± 1.3 kg/d greater ($P < 0.01$) for AA-CN compared with CTRL. Voluntary DMI and milk yield increased as lactation progressed with both treatments ($P < 0.01$). Error bars represent SE.

Arterial Concentrations

At the 3 sampling days, arterial concentrations of total EAA were increased ($P_{\text{Trt}} = 0.07$; Table 4) by AA-CN infusion. Individually, the arterial concentrations of His, Lys, and Val were greater with AA-CN compared with CTRL ($0.01 \leq P_{\text{Trt}} \leq 0.06$), concentrations of Arg, Ile, and Leu tended to be greater ($P_{\text{Trt}} \leq 0.18$), whereas Met, Thr, and Trp concentrations did not differ between treatments ($P_{\text{Trt}} \geq 0.26$). The arterial concentration of Phe was greater at 5 and 15 DIM with AA-CN but did not differ between treatments at 29 DIM ($P_{\text{Trt} \times \text{DIM}} < 0.01$). Except for His and Thr, arterial concentrations of all individual EAA increased or tended to increase with DIM ($0.01 \leq P_{\text{DIM}} \leq 0.13$), whereas the concentration of His decreased ($P_{\text{DIM}} = 0.02$) and that of Thr did not differ among DIM ($P_{\text{DIM}} = 0.94$).

The effect of treatment and DIM on arterial concentration of individual NEAA differed greatly among AA (Table 4). Most remarkably, the arterial concentration of Pro was greater with AA-CN compared with CTRL ($P_{\text{Trt}} < 0.01$), whereas the concentration of Tyr was constant with CTRL and tended to increase with AA-CN ($P_{\text{Trt} \times \text{DIM}} = 0.15$). The arterial concentration of Asp was greater with AA-CN at 5 DIM but did

Table 3. Intake, milk and protein yields, and milk protein composition¹

Item	5 DIM		15 DIM		29 DIM		SEM ²	<i>P</i> -value, fixed effects		
	CTRL	AA-CN	CTRL	AA-CN	CTRL	AA-CN		Trt	DIM	Trt × DIM
Intake										
DM diet, ³ kg/d	18.6	15.7†	21.4	19.8	19.9	23.0†	1.3	0.74	0.01	0.10
MP diet, ⁴ g/d	2,063	1,726†	2,384	2,200	2,211	2,574†	149	0.74	0.01	0.10
MP infusion, g/d	—	700	—	500	—	200	—	—	—	—
MP total, g/d	2,063	2,426	2,384	2,700	2,211	2,774	149	0.03	0.12	0.66
Milk yield										
Milk, kg/d	34.8	40.7	42.3	49.5	40.6	51.9	2.3	0.03	<0.01	0.36
CP, g/d	1,393	1,599	1,378	1,525	1,133	1,439	68	0.01	0.03	0.50
True protein, g/d	1,324	1,502	1,310	1,430	1,073	1,351	64	0.01	0.03	0.46
Casein, g/d	1,086	1,186	1,078	1,117	880	1,076	53	0.06	0.05	0.35
Milk protein content, g/kg										
CP	40	39	33	31	28	28	1.0	0.31	<0.01	0.74
True protein	38	37	31	29	26	26	1.0	0.19	<0.01	0.69
Milk protein N fractions, % of total N										
True protein	95.1	94.0	95.1	93.7	94.6	93.9	0.3	0.01	0.74	0.32
Casein	77.6	74.1	78.0	73.6	77.4	75.1	1.8	0.18	0.88	0.64
Whey	17.4	19.8	17.1	20.2	17.2	18.9	1.8	0.35	0.81	0.77
MP balance, ⁵ g/d	−571	−447	−291	−155	−64 ^z	−27 ^z	94	0.30	<0.01	0.86

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or a mixture of free AA with casein profile (AA-CN) from 1 to 29 DIM.

²SEM given for n = 5.

³Including hay; see results section.

⁴Calculated using NRC (2001).

⁵MP total supply (including infused AA) minus MP requirement calculated using NRC (2001).

* $P \leq 0.10$, † $P \leq 0.20$: difference between LSM within DIM and rows in case of $P_{\text{Trt} \times \text{DIM}} \leq 0.20$.

^z $P \geq 0.10$: no difference from zero.

Table 4. Arterial concentrations (μM , unless otherwise noted)¹

Item	5 DIM		15 DIM		29 DIM		SEM ²	<i>P</i> -value, fixed effects		
	CTRL	AA-CN	CTRL	AA-CN	CTRL	AA-CN		Trt	DIM	Trt × DIM
Blood plasma										
EAA	840	1,268	1,135	1,300	933	1,186	113	0.07	0.16	0.37
Arg	54	63	65	74	69	83	6.5	0.18	0.05	0.87
His	50	57	43	48	36	52*	3.7	0.06	0.02	0.16
Ile	110	175	174	214	143	169	23	0.14	0.01	0.64
Leu	184	291	274	309	218	267	31	0.11	0.08	0.38
Lys	51	80	76	87	63	86	6.5	0.01	0.07	0.33
Met	25	31	25	24	19	25	2.8	0.26	0.13	0.29
Phe	53	97*	57	78*	57	65	4.8	0.01	0.01	<0.01
Thr	82	92	88	82	81	96	14	0.64	0.94	0.65
Trp	36	33	45	44	41	45	3.0	0.86	0.01	0.50
Val	200	337	295	341	236	297	32	0.06	0.08	0.21
NEAA	1,341	1,383	1,250	1,221	1,134	1,287	76	0.55	0.11	0.37
Ala	173	167	182	165	185	207	20	0.98	0.26	0.46
Asn	40	38	43	45	39	51	5.3	0.56	0.40	0.37
Asp	44	80*	32	42	28	28	6.9	0.10	<0.01	0.08
Cys	92	103*	102	104	96	112*	3.7	0.05	0.19	0.12
Gln	247	244	214	248	193	248	16	0.13	0.48	0.28
Glu	46	42	53	48	48	48	2.8	0.40	0.04	0.62
Gly	459	435	371	319	270	324	40	0.89	<0.01	0.19
Pro	76	169*	81	139*	72	121*	12	<0.01	0.09	0.15
Ser	104	85	75	73	68	93†	10	0.90	0.13	0.13
Tyr	56	33*	64	43*	60	58	5.5	0.02	0.05	0.15
Total AA	2,247	2,647	2,335	2,529	2,056	2,473	162	0.14	0.51	0.67
Total protein, g/L	70.5	68.9	77.1	75.5	82.0	82.0	2.7	0.69	<0.01	0.95
Albumin, g/L	28.5	27.4	31.4	29.5	30.4	30.5	1.4	0.63	0.01	0.34
Whole blood										
Hematocrit, %	31.7	32.5	29.3	29.1	28.2	27.8	1.1	0.94	<0.01	0.25
Urea-N, mM	4.8	8.7	5.4	9.1	5.7	7.7	0.7	0.01	0.50	0.31

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or a mixture of free AA with casein profile (AA-CN) from 1 to 29 DIM.

²SEM given for n = 5.

* $P \leq 0.10$, † $P \leq 0.20$: difference between LSM within DIM and rows in case of $P_{\text{Trt} \times \text{DIM}} \leq 0.20$.

not differ between treatments by 29 DIM ($P_{\text{Trt} \times \text{DIM}} < 0.08$). The arterial concentration of Gly decreased with DIM ($P_{\text{DIM}} < 0.01$), whereas the concentration of Glu increased with DIM ($P = 0.04$).

The arterial concentration of total plasma protein and albumin did not differ between treatments ($P_{\text{Trt}} \geq 0.63$; Table 4) and increased with DIM ($P_{\text{DIM}} \leq 0.01$). The arterial concentration of urea-N was greater for AA-CN ($+3.2 \pm 0.8$ mM; $P_{\text{Trt}} = 0.01$) compared with 5.3 ± 0.3 mM for CTRL.

Net PDV Fluxes

The portal venous plasma flow did not differ between treatments ($P_{\text{Trt}} = 0.84$; Table 5) but tended ($P_{\text{DIM}} = 0.12$) to increase as lactation progressed. The net PDV release of all individual EAA was greater (His, Met, Phe, Trp, Val: $P_{\text{Trt}} \leq 0.07$) or tended to be greater (Arg, Ile, Leu, Lys, Thr: $P_{\text{Trt}} \leq 0.15$) for AA-CN compared with CTRL and did not differ among DIM ($P_{\text{DIM}} \geq 0.38$). Overall, the net PDV release of total NEAA was greater for AA-CN compared with CTRL ($P_{\text{Trt}} = 0.10$), but the pattern of individual NEAA differed. The net PDV release of Gln, Pro, and Ser increased ($P_{\text{Trt}} \leq 0.08$) and tended to increase for Ala, Asn, and Gly ($P_{\text{Trt}} \leq 0.16$) in AA-CN compared with CTRL, whereas the net PDV release of Asp did not differ between treatments ($P_{\text{Trt}} = 0.39$). We detected Trt $\times$ DIM interactions ($P_{\text{Trt} \times \text{DIM}} \leq 0.11$) for the net PDV release of Cys, Glu, and Tyr.

Net Hepatic Fluxes

Net hepatic removal (negative flux) of total EAA did not differ between treatments ($P_{\text{Trt}} = 0.37$; Table 5). Individually, the net hepatic removal of His, Met, Phe, and Trp was greater for AA-CN compared with CTRL ($P_{\text{Trt}} \leq 0.07$) and did not differ between treatments for Arg, Ile, Leu, Lys, Thr, or Val ($P_{\text{Trt}} \geq 0.31$). The net hepatic removal of individual EAA did not differ among DIM ($P_{\text{DIM}} \geq 0.22$) except for His, which decreased with DIM ($P_{\text{DIM}} = 0.07$). Individually, net hepatic removal was observed for all NEAA, except for Glu, which was released on a net basis (Table 5). Net hepatic removal of total NEAA increased ($P_{\text{Trt}} = 0.08$) for AA-CN compared with CTRL. Considering individual NEAA, the net hepatic removal of Ala, Cys, Gln, and Pro was greater ($P_{\text{Trt}} \leq 0.09$) for AA-CN than for CTRL, whereas the net hepatic release of Glu was lower ($P_{\text{Trt}} = 0.09$). For the other NEAA, net hepatic fluxes did not differ between treatments.

The fractional hepatic removal of His, Phe, and Trp (net removal relative to total influx) was greater for AA-CN compared with CTRL ($P_{\text{Trt}} \leq 0.10$; Table 6)

and did not differ between treatments for Arg, Ile, Leu, Lys, Thr, or Val ($P_{\text{Trt}} \geq 0.39$). The fractional hepatic removal of Met was greater for AA-CN at 5 DIM compared with CTRL but did not differ between treatments at 29 DIM ($P_{\text{Trt} \times \text{DIM}} = 0.10$). Among the EAA, the fractional hepatic removal of His, Met, Phe, and Thr was greater than zero ($P \leq 0.07$), whereas the fractional hepatic removals of Ile, Leu, and Val did not differ from zero ($P \geq 0.18$) for any treatment $\times$ DIM combination. The fractional hepatic removal of total NEAA increased ($P_{\text{Trt}} = 0.10$) with AA-CN, mainly due to increased ($P_{\text{Trt}} \leq 0.10$) fractional removal of Ala, Cys, and Gln.

Net Splanchnic Fluxes

The total splanchnic plasma flow did not differ between treatments ($P_{\text{Trt}} = 0.92$; Table 5) but increased as lactation progressed ($P_{\text{DIM}} = 0.03$). The net splanchnic release of total and individual EAA did not differ between treatments ($P_{\text{Trt}} \geq 0.29$). The net splanchnic release of total NEAA did not differ between treatments ($P_{\text{Trt}} = 0.64$). Individually, the net splanchnic release of Glu was lower ($P_{\text{Trt}} = 0.10$) and that of Pro was greater ($P_{\text{Trt}} = 0.03$) in AA-CN compared with CTRL, whereas the net splanchnic releases of other NEAA did not differ between treatments ($P_{\text{Trt}} \geq 0.38$).

Net Mammary Fluxes

Mammary plasma flow did not differ between treatments ($P_{\text{Trt}} = 0.79$; Table 5) and tended ($P_{\text{DIM}} = 0.19$) to decrease as lactation progressed. The net mammary uptake (negative flux) of individual EAA was greater with AA-CN compared with CTRL ($P_{\text{Trt}} \leq 0.08$) except for Trp ($P_{\text{Trt}} = 0.35$). For Gln, Pro, and Tyr, net mammary uptake was greater ($P_{\text{Trt}} \leq 0.07$) and for Asn, tended to be greater ($P_{\text{Trt}} = 0.19$) with AA-CN compared with CTRL, whereas for Asp, net mammary uptake was greater with AA-CN compared with CTRL at 5 DIM ($P_{\text{Trt} \times \text{DIM}} = 0.04$). For the other NEAA, net mammary uptake did not differ between treatments ($P_{\text{Trt}} \geq 0.23$).

The ratio of mammary U:O in milk true protein of individual EAA other than Arg, Leu, and Lys did not differ between treatments ($P_{\text{Trt}} \geq 0.28$; Table 7). For Arg, mammary U:O was greater for AA-CN ($P_{\text{Trt}} = 0.10$). The mammary U:O ratio of Arg was greater than unity ($P \leq 0.01$), averaging 2.40 ± 0.04 at 5, 15, and 29 DIM. At 5 DIM, the mammary U:O ratio of Lys did not differ from unity for CTRL ($P = 0.63$) but was greater than unity for AA-CN ($P \leq 0.01$), whereas at 15 and 29 DIM, the U:O ratio was greater than unity ($P \leq 0.04$) with both treatments ($P_{\text{Trt} \times \text{DIM}} = 0.02$).

Table 5. Splanchnic and mammary plasma flows (L/h) and net fluxes of EAA and NEAA (mmol/h)¹

Item	Site ²	5 DIM		15 DIM		29 DIM		SEM ³	<i>P</i> -value, fixed effects		
		CTRL	AA-CN	CTRL	AA-CN	CTRL	AA-CN		Trt	DIM	Trt × DIM
Plasma flow	PDV	1,221	1,201	1,424	1,336	1,372	1,439	94	0.84	0.12	0.71
	TSP	1,342	1,285	1,564	1,546	1,475	1,586	121	0.92	0.03	0.68
	MG	896	871	791	795	839	785	93	0.79	0.19	0.93
EAA	PDV	208	277	264	245	231	298	26	0.06	0.71	0.38
	HEP	−30	−92	−47	−46	−47	−58	23	0.37	0.57	0.30
	TSP	170	172	198	194	188	238	31	0.67	0.41	0.67
	MG	−237	−276	−236	−271	−198	−263	14	0.01	0.31	0.62
	Milk	204	231	202	220	165	208	10	0.01	0.03	0.46
Arg	PDV	20	26	24	26	21	29	2.8	0.12	0.63	0.45
	HEP	−1.4	−6.9	−5.6	−6.5	−5.0	−5.6	2.3	0.44	0.56	0.35
	TSP	17	18	22	19	16	24	3.5	0.65	0.65	0.31
	MG	−28	−31	−23	−30	−22	−29	1.7	0.01	0.10	0.55
	Milk	11	13	11	12	9.1	11	0.5	0.01	0.03	0.46
His	PDV	9.4	13	10	12	9.0	14	1.1	0.02	0.95	0.58
	HEP	−4.3	−9.7	−3.3	−6.3	−3.3	−6.2	1.2	0.02	0.07	0.44
	TSP	5.0	2.6	6.9	6.0	5.7	7.3	1.6	0.76	0.13	0.47
	MG	−10	−11	−10	−11	−9.1	−11	0.6	0.05	0.33	0.81
	Milk	9.9	11	9.8	11	8.0	10	0.5	0.01	0.03	0.46
Ile	PDV	24	28	25	24	24	34	3.1	0.11	0.38	0.28
	HEP	1.3	−3.0	0.6	1.4	−1.7	−0.3	2.7	0.83	0.41	0.40
	TSP	25	24	26	26	22	33	3.6	0.42	0.55	0.22
	MG	−31	−36	−30	−34	−25	−34	1.9	0.01	0.32	0.51
	Milk	25	29	25	27	20	26	1.2	0.01	0.03	0.46
Leu	PDV	42	48	45	42	40	59*	5.3	0.14	0.44	0.19
	HEP	0.4	−6.3	0.3	0.1	−3.5	−1.0	4.3	0.78	0.51	0.50
	TSP	42	41	45	43	37	57*	6.1	0.45	0.54	0.13
	MG	−50	−59	−49	−55	−41	−56	3.0	0.01	0.29	0.33
	Milk	42	47	41	45	34	43	2.0	0.01	0.03	0.46
Lys	PDV	27	38	33	36	31	40	4.0	0.14	0.81	0.51
	HEP	−2.8	−6.8	−1.8	−2.7	−5.0	−1.9	2.8	0.87	0.34	0.35
	TSP	24	30	31	34	27	37	4.6	0.29	0.29	0.57
	MG	−32	−45*	−35	−40*	−30	−39*	1.7	<0.01	0.08	0.12
	Milk	32	36	32	34	25.8	33	1.5	0.01	0.03	0.46
Met	PDV	11	17	14	15	14	17	1.7	0.02	0.65	0.47
	HEP	−2.9	−11*	−4.8	−6.0	−5.3	−6.2	1.6	0.07	0.55	0.12
	TSP	7.8	6.8	10	8.7	10	11	1.5	0.74	0.18	0.87
	MG	−11	−15	−13	−13	−10	−12	0.8	0.04	0.09	0.26
	Milk	11	12	11	12	8.8	11	0.5	0.01	0.03	0.46
Phe	PDV	24	37	28	31	25	34	3.1	0.03	0.92	0.23
	HEP	−11	−24*	−14	−19†	−13	−19†	2.4	0.02	0.73	0.11
	TSP	13	12	14	12	11	15	2.5	0.91	0.92	0.41
	MG	−20	−22	−19	−20	−16	−19	1.1	0.08	0.03	0.79
	Milk	17	19	17	18	14	17	0.8	0.01	0.03	0.46
Thr	PDV	23	29	26	27	23	32	3.3	0.15	0.85	0.46
	HEP	−8.5	−15	−9.7	−10	−10	−13	2.7	0.31	0.57	0.40
	TSP	15	14	16	16	13	19	2.8	0.64	0.69	0.48
	MG	−22	−25	−21	−24	−18	−22	1.2	0.01	0.03	0.79
	Milk	21	23	20	22	17	21	1.0	0.01	0.03	0.46

Continued

Table 5 (Continued). Splanchnic and mammary plasma flows (L/h) and net fluxes of EAA and NEAA (mmol/h)¹

Item	Site ²	5 DIM		15 DIM		29 DIM		SEM ³	<i>P</i> -value, fixed effects		
		CTRL	AA-CN	CTRL	AA-CN	CTRL	AA-CN		Trt	DIM	Trt × DIM
Trp	PDV	4.2	7.2	5.9	7.1	5.6	7.2	0.9	<0.01	0.63	0.57
	HEP	0.6	−3.3	−3.5	−2.9	−1.6	−4.9	1.0	0.01	0.22	0.24
	TSP	4.1	3.2	2.4	3.5	4.5	3.0	1.0	0.66	0.86	0.46
	MG	−5.0	−6.2	−5.4	−6.3	−3.8	−4.8	0.9	0.35	0.23	0.98
	Milk	4.1	4.6	4.0	4.4	3.3	4.2	0.2	0.01	0.03	0.46
Val	PDV	24	27	24	24	22	34*	3.1	0.07	0.42	0.17
	HEP	1.3	−5.2	−0.7	−0.8	−0.2	−2.3	3.3	0.46	0.82	0.48
	TSP	24	21	25	23	22	31†	3.6	0.70	0.49	0.20
	MG	−30	−35	−31	−35	−27	−34	2.0	0.05	0.42	0.72
	Milk	31	36	31	34	25	32	1.5	0.01	0.03	0.46
NEAA	PDV	241	300	200	257	218	312	31	0.10	0.37	0.86
	HEP	−163	−261	−113	−206	−172	−229	31	0.08	0.08	0.71
	TSP	89	26	81	51	53	80	35	0.64	0.81	0.39
	MG	−180	−189	−151	−179	−117	−155	15	0.22	0.04	0.68
	Milk	252	286	250	273	205	258	12	0.01	0.03	0.46
Ala	PDV	65	78	75	78	66	96	9.4	0.14	0.60	0.34
	HEP	−60	−93	−60	−75	−55	−79	10	0.09	0.37	0.42
	TSP	5.2	−16	16	3.3	11	18	11	0.53	0.06	0.28
	MG	−30	−23	−27	−20	−24	−23	4.4	0.25	0.79	0.80
	Milk	21	24	21	22	17	21	1.0	0.01	0.03	0.46
Asn	PDV	25	30	28	29	25	35	3.3	0.15	0.74	0.35
	HEP	−13	−16	−15	−18	−17	−22	2.6	0.28	0.13	0.94
	TSP	12	13	13	12	7.4	14	3.1	0.52	0.75	0.36
	MG	−14	−15	−11	−13	−9.8	−13	1.3	0.19	0.10	0.63
	Milk	17	20	17	19	14	18	0.8	0.01	0.03	0.46
Asp	PDV	12	9.6	6.5	4.6	8.2	5.1	2.5	0.39	0.16	0.97
	HEP	−10	4.2	−10	−11	−11.2	−8.5	4.7	0.27	0.19	0.38
	TSP	1.6	12	−3.2	−6.3	−1.9	−2.5	3.3	0.52	0.01	0.24
	MG	−5.4	−21*	−6.3	−9.4	−4.6	−5.3	2.5	0.05	0.01	0.04
	Milk	15	17	15	16	12	15	0.7	0.01	0.03	0.46
Cys	PDV	3.2	2.1	0.7	2.3	0.9	4.9*	1.0	0.11	0.32	0.08
	HEP	−3.1	−5.0	−4.5	−6.5	−4.4	−7.3	1.1	0.04	0.31	0.90
	TSP	−0.3	−3.2	−3.5	−4.2	−3.5	−2.6	1.2	0.49	0.22	0.37
	MG	−4.3	−4.3	−3.9	−3.8	−3.5	−2.5	0.9	0.73	0.25	0.73
	Milk	3.4	3.9	3.4	3.7	2.8	3.5	0.2	0.01	0.03	0.46
Gln	PDV	1.2	31*	2.9	19*	3.4	22*	4.7	0.01	0.29	0.20
	HEP	6.9	−21*	4.5	−15*	−0.9	−13*	3.7	<0.01	0.81	0.20
	TSP	8.1	10	7.9	4.2	2.3	8.5	4.5	0.75	0.69	0.49
	MG	−54	−59	−37	−41	−28	−37	3.2	0.07	<0.01	0.76
	Milk	36	41	35	39	29	36	1.7	0.01	0.03	0.46
Glu	PDV	4.4	3.3	3.5	4.6	3.2	7.4*	1.3	0.40	0.25	0.11
	HEP	34	22	38	33	34	28	3.6	0.09	0.05	0.58
	TSP	38	25	42	36	37	35	3.5	0.10	0.08	0.29
	MG	−25	−20	−25	−23	−24	−23	2.1	0.23	0.65	0.58
	Milk	47	53	47	51	38	48	2.3	0.01	0.03	0.46
Gly	PDV	31	32	21	28	26	38	4.3	0.16	0.11	0.47
	HEP	−69	−76	−54	−60	−59	−70	7.7	0.41	0.04	0.90
	TSP	−40	−46	−32	−31	−30	−32	7.6	0.87	0.08	0.78
	MG	4.3	−0.2	−0.1	−9.1	0.8	−1.6	4.0	0.30	0.07	0.50
	Milk	14	16	14	15	11	14	0.7	0.01	0.03	0.46

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Continued

Table 5 (Continued). Splanchnic and mammary plasma flows (L/h) and net fluxes of EAA and NEAA (mmol/h)¹

Item	Site ²	5 DIM		15 DIM		29 DIM		SEM ³	<i>P</i> -value, fixed effects		
		CTRL	AA-CN	CTRL	AA-CN	CTRL	AA-CN		Trt	DIM	Trt × DIM
Pro	PDV	20	37	20	31	18	30	2.5	<0.01	0.22	0.39
	HEP	−8	−19	−8.5	−15	−9.9	−15	2.5	0.05	0.53	0.40
	TSP	11	18	12	16	8.4	15	2.2	0.03	0.33	0.69
	MG	−17	−24	−14	−19	−11	−14	1.3	<0.01	<0.01	0.53
Ser	Milk	49	55	48	52	39	50	2.3	0.01	0.03	0.46
	PDV	35	46	39	44	36	48	4.4	0.08	0.95	0.63
	HEP	−30	−41	−31	−37	−34	−42	5.7	0.26	0.60	0.86
	TSP	4.6	4.5	8.1	6.1	2.2	6.0	5.6	0.94	0.52	0.62
Tyr	MG	−14	−19	−19	−22	−13	−19	3.2	0.25	0.14	0.82
	Milk	34	39	34	37	27	35	1.6	0.01	0.03	0.46
	PDV	19	15	22	18	20	26†	2.4	0.77	0.08	0.11
	HEP	−9.5	−5.3	−11	−7.4	−12	−13	1.5	0.15	0.02	0.24
Total AA	TSP	9.3	9.7	11	11	8.0	13	1.9	0.38	0.66	0.30
	MG	−14	−17	−15	−16	−12	−16	0.9	0.02	0.14	0.25
	Milk	17	20	17	19	14	18	0.8	0.01	0.03	0.46
	PDV	469	586	426	510	465	608	58	0.12	0.48	0.93
	HEP	−198	−355	−108	−251	−218	−283	50	0.12	0.03	0.48
	TSP	291	199	285	256	250	319	67	0.84	0.69	0.55
	MG	−430	−465	−389	−452	−319	−418	25	0.04	0.07	0.54
	Milk	456	517	451	493	370	466	22	0.01	0.03	0.46

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or a mixture of free AA with casein profile (AA-CN) from 1 to 29 DIM.²PDV = portal-drained viscera; HEP = hepatic tissue; TSP = total splanchnic tissue; MG = mammary gland.³SEM given for n = 5.* $P \leq 0.10$, † $P \leq 0.20$: difference between LSM within DIM and rows in case of $P_{\text{Trt} \times \text{DIM}} \leq 0.20$.

Table 6. Hepatic fractional removal of AA as percentage of total hepatic influx¹

AA	5 DIM		15 DIM		29 DIM		SEM ²	<i>P</i> -value, fixed effects		
	CTRL	AA-CN	CTRL	AA-CN	CTRL	AA-CN		Trt	DIM	Trt × DIM
EAA	2.0 ^z	4.8	2.2 ^z	2.2	3.1	2.8	1.2	0.52	0.43	0.43
G1 EAA ³	7.5	13	8.1	9.1	9.1	10	1.4	0.11	0.35	0.25
G2 EAA ³	−0.3 ^z	1.6 ^z	0.2 ^z	0.3 ^z	1.3 ^z	0.5 ^z	1.0	0.73	0.70	0.37
Arg	1.5 ^z	6.3	4.6	5.0	4.7	4.0	2.0	0.53	0.88	0.35
His	5.8	11	4.1	7.6	6.1	6.5	1.5	0.10	0.12	0.29
Ile	−1.1 ^z	1.2 ^z	−0.04 ^z	−0.1 ^z	1.2 ^z	0.3 ^z	1.0	0.72	0.64	0.28
Leu	−0.5 ^z	1.4 ^z	0.1 ^z	0.2 ^z	1.3 ^z	0.4 ^z	1.1	0.74	0.72	0.42
Lys	2.4 ^z	4.6	1.1 ^z	1.9 ^z	5.2	1.2 ^z	2.0	0.89	0.23	0.18
Met	6.6	18*	8.8	12	13	11	2.7	0.18	0.63	0.10
Phe	10	15	12	13	12	14	1.3	0.10	0.61	0.35
Thr	6.0	9.6†	5.8	7.3	9.0	7.0	1.7	0.63	0.36	0.19
Trp	−0.6 ^z	6.8*	4.6	3.8	2.1 ^z	5.9†	1.4	<0.01	0.89	0.11
Val	−0.6 ^z	1.1 ^z	0.2 ^z	0.2 ^z	0.3 ^z	0.6 ^z	0.7	0.39	0.93	0.49
NEAA	7.4	12	6.1	9.6	9.4	9.9	1.3	0.10	0.27	0.34
Ala	20	32	18	23	17	19	2.9	0.07	0.04	0.30
Asn	16	21	16	19	24	19	3.3	0.79	0.45	0.25
Asp	11 ^z	−1.5 ^z	15	14	17	17	5.5	0.49	0.09	0.54
Cys	2.5	3.8	2.9	4.0	3.2	4.2	0.7	0.09	0.75	0.98
Gln	−1.8 ^z	6.0*	−1.4 ^z	4.2*	0.9 ^z	3.4†	1.0	<0.01	0.72	0.10
Glu	−52	−40	−45	−40	−48	−34	4.7	0.09	0.58	0.32
Gly	11	13	9.7	12	16	13	1.9	0.80	0.07	0.25
Pro	6.6	7.1	5.8	6.1	9.8	6.8	1.3	0.60	0.16	0.31
Ser	17	27†	21	23	28	21	3.6	0.74	0.62	0.09
Tyr	10	9.0	9.3	9.3	11	11	1.6	0.76	0.36	0.90
Total AA	4.9	8.6	3.4	5.8	6.7	6.4	1.2	0.23	0.09	0.28

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or a mixture of free AA with casein profile (AA-CN) from 1 to 29 DIM.²SEM given for n = 5.³Group (G)1 EAA = His, Met, Phe, Tyr, and Trp; G2 EAA = Ile, Leu, Lys, and Val.* $P \leq 0.10$, † $P \leq 0.20$: difference between LSM within DIM and rows in case of $P_{\text{Trt} \times \text{DIM}} \leq 0.20$.^z $P \geq 0.10$: fractional hepatic uptake not different from zero.

Table 7. Mammary uptake to milk output ratio of AA as well as net PDV release, net TSP release, and MG uptake to milk output ratios of AA-N^{1,2}

AA	5 DIM		15 DIM		29 DIM		SEM ³	P-value, fixed effects		
	CTRL	AA-CN	CTRL	AA-CN	CTRL	AA-CN		Trt	DIM	Trt × DIM
Mammary uptake to milk output ratio										
EAA	1.16	1.19	1.15	1.23	1.23	1.26	0.03	0.10	0.16	0.78
G1 EAA ⁴	1.00 ^u	1.04	1.04 ^u	1.06	1.02 ^u	1.05	0.02	0.26	0.49	0.95
G2 EAA ⁴	1.09	1.18	1.13	1.17	1.18	1.23	0.03	0.02	0.18	0.82
Arg	2.49	2.46	2.06	2.46	2.43	2.50	0.11	0.10	0.25	0.24
His	1.03 ^u	1.02 ^u	1.04 ^u	1.04 ^u	1.11	1.07	0.04	0.63	0.35	0.88
Ile	1.22	1.25	1.20	1.25	1.26	1.32	0.05	0.28	0.37	0.96
Leu	1.19	1.24	1.20	1.21	1.22	1.31	0.04	0.19	0.38	0.75
Lys	1.02 ^u	1.25*	1.09	1.16	1.16	1.21	0.03	0.01	0.17	0.02
Met	1.05 ^u	1.19†	1.16	1.10	1.09 ^u	1.10	0.06	0.66	0.81	0.13
Thr	1.06	1.08	1.03 ^u	1.05	1.04	1.04	0.02	0.52	0.22	0.59
Trp	1.20 ^u	1.34	1.30 ^u	1.41	1.13 ^u	1.12 ^u	0.17	0.70	0.31	0.91
Val	0.96 ^u	0.97 ^u	1.01 ^u	1.04 ^u	1.07 ^u	1.06 ^u	0.04	0.82	0.14	0.87
NEAA	0.69	0.66	0.59	0.65	0.58	0.60	0.05	0.84	0.35	0.64
Ala	1.45	0.96 ^u	1.30 ^u	0.87 ^u	1.41	1.06 ^u	0.20	0.06	0.68	0.95
Asn	0.82	0.74	0.65	0.70	0.68	0.71	0.05	0.99	0.23	0.51
Asp	0.37	1.23* ^u	0.42	0.60	0.40	0.35	0.16	0.11	0.03	0.06
Cys	1.16 ^u	1.11 ^u	1.16 ^u	1.01 ^u	1.28 ^u	0.69 ^u	0.25	0.41	0.72	0.44
Gln	1.50	1.46	1.03 ^u	1.05 ^u	0.97 ^u	1.02 ^u	0.05	0.81	<0.01	0.70
Glu	0.55	0.38	0.56	0.46	0.63	0.48	0.05	0.02	0.21	0.69
Gly	−0.46	0.01	0.002	0.59 ^u	−0.13	0.10	0.32	0.26	0.14	0.79
Pro	0.35	0.43	0.30	0.37	0.26	0.29	0.02	<0.01	<0.01	0.25
Ser	0.43	0.50	0.55	0.59	0.46	0.55	0.09	0.54	0.17	0.90
Total AA	0.90	0.90	0.86	0.91	0.88	0.90	0.03	0.57	0.97	0.68
Net flux to milk output ratio										
EAA-N										
PDV release	1.08 ^u	1.28	1.44	1.23	1.55	1.53	0.12	0.97	0.08	0.41
TSP release	0.85 ^u	0.79 ^u	1.14 ^u	0.93 ^u	1.23 ^u	1.20 ^u	0.14	0.50	0.09	0.83
MG uptake	1.29	1.32	1.25	1.36	1.37	1.39	0.04	0.11	0.22	0.55
G1 EAA-N ³										
PDV release	1.06 ^u	1.27	1.46	1.28	1.51	1.56	0.13	0.65	0.06	0.51
TSP release	0.57	0.43	0.84 ^u	0.64	0.84 ^u	0.78	0.13	0.32	0.11	0.87
MG uptake	1.01 ^u	1.03 ^u	1.04 ^u	1.06	1.03 ^u	1.05	0.03	0.50	0.57	0.99
G2 EAA-N ³										
PDV release	0.91 ^u	0.96 ^u	1.03 ^u	0.95 ^u	1.13 ^u	1.24	0.11	0.80	0.11	0.64
TSP release	0.85 ^u	0.79	1.01 ^u	0.91 ^u	1.01 ^u	1.15 ^u	0.11	0.92	0.13	0.56
MG uptake	1.07	1.19	1.12	1.17	1.17	1.22	0.03	<0.01	0.14	0.53
NEAA-N										
PDV release	0.90 ^u	1.05 ^u	0.67 ^u	0.96 ^u	1.03 ^u	1.19 ^u	0.13	0.30	0.08	0.80
TSP release	0.37	0.14	0.35	0.19	0.25	0.33	0.12	0.52	0.80	0.43
MG uptake	0.78	0.75	0.65	0.70	0.64	0.66	0.05	0.90	0.13	0.70
Total AA-N										
PDV release	1.02 ^u	1.17 ^u	1.00 ^u	1.10 ^u	1.31	1.35	0.13	0.49	0.21	0.94
TSP release	0.66 ^u	0.46	0.77 ^u	0.58	0.76 ^u	0.76 ^u	0.14	0.43	0.40	0.77
MG uptake	1.03 ^u	1.02 ^u	0.97 ^u	1.02 ^u	1.00 ^u	1.01 ^u	0.02	0.62	0.72	0.62

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or a mixture of free AA with casein profile (AA-CN) from 1 to 29 DIM.²PDV = portal-drained viscera; HEP = hepatic tissue; TSP = total splanchnic tissue; MG = mammary gland.³SEM given for n = 5.⁴Group (G)1 EAA = His, Met, Phe, Tyr, and Trp; G2 EAA = Ile, Leu, Lys, and Val.**P* ≤ 0.10, †*P* ≤ 0.20: difference between LSM within DIM and rows in case of *P*_{Trt × DIM} ≤ 0.20.^u*P* ≥ 0.10: uptake to output ratios was not different from unity (1.00).

The mammary U:O ratio of Ile and Leu was greater than unity ($P \leq 0.01$) for all treatment $\times$ DIM combinations, whereas for Val, the ratio did not differ from unity at any time ($P \geq 0.17$). The U:O ratio of total NEAA was below unity ($P \leq 0.01$) with all treatment $\times$ DIM combinations and did not differ between treatments ($P_{\text{Trt}} = 0.84$). The mammary U:O ratio of Ala decreased ($P_{\text{Trt}} = 0.06$), whereas that of Pro increased ($P_{\text{Trt}} < 0.01$) in AA-CN compared with CTRL. The ratio between net splanchnic releases of total AA-N and output in milk protein yield was lower than unity ($P \leq 0.02$) for AA-CN at 5 and 15 DIM, whereas for CTRL, the ratio did not differ from unity ($P \geq 0.11$) at any DIM.

DISCUSSION

The main outcome of the current investigation was that, in the early postpartum period, abomasal infusion of AA greatly increased milk and protein yields with very high marginal utilization of the extra supplied AA to support the increased milk protein yield. It was striking to note the close similarity of these results to the observations by Larsen et al. (2014b) with abomasal casein infusion in the similar early postpartum period. Thus, similar responses in milk production in early postpartum dairy cows were obtained by abomasal infusion of casein or free AA combined with casein profile. Previously, it was observed that milk protein yield was greater with abomasal infusion of casein or casein hydrolysate compared with a corresponding AA mixture (Choung and Chamberlain, 1995), indicating a potential role for bioactive peptides absorbed from the small intestine. However, Galindo et al. (2011) did not observe this when comparing abomasal infusion of casein or free AA in mid-lactation dairy cows, in line with the close similarity of the current observations and those of Larsen et al. (2014b) in postpartum dairy cows.

The greater milk production with abomasal AA infusion was generally not supported by greater DMI except in the last period of sampling. This result somewhat contrasts a previous observation with dairy cows in the first 4 wk of lactation where abomasal infusion of casein did not affect DMI (Larsen et al., 2014b). However, in mid-lactation dairy cows, abomasal infusion of both casein and corresponding AA mixtures have not been observed to decrease voluntary DMI (Choung and Chamberlain, 1995; Galindo et al., 2011). It could be speculated that the presence of one or more specific free AA in the abomasum and proximal duodenum may have affected intake via gut hormones, the vagus nerve, or both, as interpreted from studies of AA effects on intake regulation in humans and rats (Fromentin et al., 2012; San Gabriel and Uneyama, 2013).

PDV Metabolism of AA

We hypothesized that abomasal infusion of AA would increase the net PDV release of all individual EAA, as was observed. However, it appears that the increases in net PDV release of His, Met, Phe, and Trp were relatively more pronounced than those of the branched-chain AA (Ile, Leu, and Val) and Lys. The estimated net PDV recoveries of infused individual EAA (corrected for treatment differences in DMI, see calculations) ranged from 69 to 73% for the branched-chain AA and Lys, and from 72 to 102% for His, Met, Phe, and Thr (Table 8). This observed ranking of net PDV recoveries of individual EAA was in line with previous observations for sheep (MacRae et al., 1997; El-Kadi et al., 2006) and dairy cows (Pacheco et al., 2006; Reynolds, 2006). Therefore, the current experiment would indicate limited extra PDV metabolism of His, Met, Phe, and Thr in AA-CN compared with CTRL cows. This would suggest no limitation from these AA to the postpartum proliferation of gastrointestinal tissues (Gibb et al., 1992; Reynolds et al., 2004) or endogenous protein secretion into the digestive tract. In contrast, because the low PDV recoveries were confined to the branched-chain AA, this would suggest that these AA were catabolized by the PDV to a higher extent with increased supply as previously reported in dairy cows

Table 8. Net portal-drained viscera (PDV) recovery (%) of abomasally infused AA averaged over 5, 15, and 29 DIM¹

Item	Mean	SE
EAA	73	26
Arg	131	32
His	102	26
Ile	73	47
Leu	69	42
Lys	71	13
Met	91	20
Phe	72	14
Thr	79	25
Trp	231	49
Val	70	44
NEAA	105	33
Ala	289	123
Asn	120	40
Asp	−93	78
Cys	290	260
Gln	114	40
Glu	43	35
Gly	116	66
Pro	72	9
Ser	88	15
Tyr	—	—
Total AA	99	31

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or mixture of free AA with casein profile (AA-CN) from 1 to 29 DIM; calculated from the mean net PDV release corrected for changes in DMI observed for each treatment on each sampling day; see text for details of the calculations.

(Lapierre et al., 2002). Interestingly, the individual EAA with highest and lowest net PDV recoveries, respectively, coincided somewhat with the grouping the EAA according to patterns of mammary and hepatic metabolism (group 1: His, Met, Phe, Trp, and Tyr; group 2: Ile, Leu, Val, and Lys; Mephram, 1982; Lapierre et al., 2005), in agreement with the distribution of enzymes responsible of AA catabolism (Lobley and Lapierre, 2003).

The abomasal infusion of AA increased the net PDV release of all individual NEAA with the exception of Asp, Glu, and Tyr. On a total basis, the net PDV recovery of infused NEAA was essentially 100% (Table 8), but indeed, this average covered a range from about -100 to +300%, with Asp and Ala representing the extremes. Concerning Ala, the >250% net PDV recovery of infused Ala was in line with transamination of amino groups from catabolized AA to pyruvate (Reynolds, 2006). The relatively low net PDV release of Glu and Tyr at 5 DIM with AA-CN was likely caused by the partial or complete replacement of Glu and Tyr in the AA infusate due to solubility constraints. Aspartate behaved differently, as its net PDV release was numerically lower in AA-CN compared with CTRL cows on the 3 sampling days. The small intestine exerts extensive catabolism of Asp, Glu, and Gln in mammals (Windmueller and Spaeth, 1980; Lobley and Lapierre, 2003) in agreement with the current close-to-zero net PDV fluxes of Asp and Glu. For Gln, it was interesting to note that, although the net PDV release of Gln was essentially zero with CTRL, the net PDV release of Gln was increased on average by 22 mmol/h for AA-CN. The estimated average net PDV recovery of infused Gln was 114% (Table 8). Such a high net PDV recovery of Gln was also observed in dairy cows abomasally infused with Gln as the sole AA (Doepel et al., 2007) or in sheep infused with casein (El-Kadi et al., 2006). These observations may indicate saturation of metabolic pathways utilizing Gln in the PDV tissues.

Hepatic Metabolism of AA

The greater net PDV release of His, Met, Phe, and Trp with abomasal infusion of AA was associated with equivalently greater net hepatic removal of these EAA, often averaging close to 50% of the net PDV release. This resulted in the net release from total splanchnic tissues being unaffected for these EAA by AA-CN infusion. The hepatic removal of group 1 AA is usually related to total influx (Hanigan, 2005; Lapierre et al., 2005). However, this ratio tended to increase with abomasal AA infusion in the current experiment, indicating that hepatic catabolic pathways may be reduced in postpartum cows experiencing the typical postpartum

AA deficiency (Bell et al., 2000). The hepatic removal of the branched-chain AA (Ile, Leu, and Val) and Lys (the group 2 EAA) is usually limited (Hanigan, 2005; Lapierre et al., 2005). In line with this pattern, the hepatic removal of group 2 AA was limited with both treatments; further, when expressed relative to total influx, hepatic removal did not differ from zero.

Generally, AA removed by the liver can be used for several anabolic processes; for example, synthesis of export proteins (e.g., albumin, peptide hormones, and immune proteins) and constitutive proteins, as well as for catabolic processes such as gluconeogenesis and complete oxidation. The partitioning of AA among these metabolic pathways cannot be assessed in the current experiment and requires application of isotopic labeling techniques. Liver proliferation is known to occur in the period investigated (Gibb et al., 1992; Reynolds et al., 2004) and would thus have required sequestration of AA to support gain of constitutive hepatic proteins in the current experiment. To which extent liver proliferation was greater with AA-CN is unknown; however, it would likely have been of limited relative importance because the general postpartum liver CP proliferation is estimated to be 8 g/d based on Gibb et al. (1992), equivalent to 4 mmol of AA-N/h. The basal turnover of liver constitutive proteins would not affect net flux measurements. In addition, liver synthesis of plasma albumin requires AA. Larsen et al. (2014a) measured the liver synthesis of plasma albumin to be 25 and 30 g/d at 4 and 29 DIM, respectively, in dairy cows fed a diet with a normal CP level, and reported that synthesis was increased by 5 g/d during early lactation in cows abomasally infused with casein. These syntheses would be equivalent to 12 and 14 mmol of AA-N/h, respectively, and the increment to approximately 2.5 mmol of AA-N/h. Overall, these calculations indicate that the requirement of AA for additional synthesis of these major proteins induced by the greater postpartum AA availability with AA-CN is of limited quantitative importance.

The increase in arterial urea-N with AA-CN at 5 DIM was tremendous: $+3.9 \pm 1.1$ mM, and was similar to the increase observed by Larsen et al. (2014b) with abomasal casein infusion. The greater circulating levels of urea indicate use in the liver of AA for gluconeogenesis or oxidation, or a combination thereof. The glucogenic AA (excluding ketogenic Leu and Lys) removed by the liver at 5 DIM could maximally have contributed 97 and 171 mmol/h of glucose synthesis for CTRL and AA-CN, respectively, assuming catabolism of 2 AA molecules for the synthesis of 1 glucose molecule. Although the greater hepatic removal of glucogenic AA with AA-CN could support a maximal increase in net hepatic glucose release of 74 mmol/h, the measured

true hepatic glucose release was unaffected by greater AA supply, as shown in the companion paper (Galindo et al., 2015).

Mammary AA Metabolism

The mammary uptake of EAA increased to support the increased milk output, to the same extent as milk output for group 1 AA (except Trp) and to a greater extent for Arg, Leu, and Lys. The increase of the mammary U:O ratio of Lys was the most significant: at 5 DIM it did not differ from unity for CTRL but was greater than unity for AA-CN. This difference in the first week postpartum was in line with Larsen et al. (2014b) and indicates that, with typical European or American diets, Lys maybe one of the most limiting AA in the early postpartum period. Indeed, a Lys deficiency decreased the U:O ratio to minimize the effect of a reduced supply on milk protein yield (Lapierre et al., 2009).

On a total N basis, the mammary gland was in N balance; that is, the mammary uptake of total AA-N was equivalent to the N output in milk protein for all treatment $\times$ DIM combinations. This indicates transfer of N from AA taken up in excess (i.e., Arg, the branched-chain AA, Lys, Ala, and Gln) to support mammary synthesis of the NEAA not taken up in sufficient amounts to support milk protein output in the early postpartum period, similar to observations in the galactopoietic period (Lapierre et al., 2009). Further, the mammary U:O ratio of Ala was greater for CTRL, indicating an important role of Ala as an inter-organ N shuttle in situations of dietary protein deficiency, as previously observed by Larsen et al. (2014b) and Doepel and Lapierre (2010).

Whole-Body AA Metabolism

The negative whole-body MP balance typically observed in the early postpartum period (Grummer, 1995; Bell et al., 2000) was not clearly diminished in the current study, even though the MP supply was increased by 19% with AA-CN when averaged over 5, 15, and 29 DIM. The high marginal utilization of infused AA for milk CP, averaging 55%, contrasts the average of 21% reported for casein infusion in cows in established lactation (Hanigan et al., 1998), and indicates a high demand by the mammary gland in the early postpartum period. Indeed, the constant efficiency of utilization of MP supply to cover maintenance and milk protein requirements used in many feeding systems, including NRC (2001), may not apply to the early postpartum dairy cow. Assuming that the inefficiency of utilization of EAA can be calculated as the sum of AA catabolism

relative to the digestive flow, the efficiency of utilization of EAA would effectively not be fixed and be higher than the fixed efficiency currently used (0.67), averaging 0.75, 0.74, and 0.71 for CTRL cows at 5, 15, and 29 DIM, respectively.

The estimated changes in PDV, hepatic, and mammary use of EAA in response to abomasal AA infusion (Figure 2) may reflect the coordination of AA metabolism between tissues contributing to whole-body balance of EAA. The observed treatment differences in DMI, however, somewhat bias these calculations; therefore, the changes with AA-CN were corrected for the differences in DMI. Considering the overall fate of the infused group 1 EAA (Figure 2a; His, Met, Phe, Trp, and Tyr) and group 2 EAA (Figure 2b; Ile, Leu,

Figure 2. Estimated net use (negative flux) of abomasally infused group 1 EAA (a; His, Met, Phe, Tyr, and Trp) and group 2 EAA (b; Ile, Leu, Lys, and Val) partitioned among portal-drained visceral (PDV), hepatic (HEP), and mammary (MG) tissues at 5, 15, and 29 DIM. The change in net PDV, hepatic, and mammary flux due to abomasal AA infusion with AA-CN was estimated by subtracting the net fluxes with dietary origin from the measured net PDV, hepatic, and mammary flux with AA-CN, respectively, corrected for treatment differences in DMI. The balance was estimated by difference between AA abomasally infused and summated increase in PDV, hepatic and mammary use with AA-CN.

Lys, and Val) with AA-CN, the PDV use of group 1 EAA was generally not increased by AA-CN infusion, whereas the PDV use of group 2 EAA was increased, reflecting the previously discussed net PDV recoveries of EAA. The hepatic and mammary use of both group 1 and 2 EAA was increased by infusion, particularly at 5 DIM, whereas it was essentially not increased by 29 DIM. Summing the increased use of group 1 and 2 EAA in PDV, hepatic, and mammary tissues with AA-CN at 5 DIM, this adds to 54 and 106 mmol/h, respectively, compared with 2.9 and 3.6 mmol/h, respectively, at 29 DIM. The use of group 1 and 2 EAA at 5 DIM was considerably greater than the infused amounts (34 and 68 mmol/h; Table 2). Hence, increasing the early postpartum MP supply actually seemed to increase the postpartum MP deficiency in the first week postpartum, whereas the greater mammary AA uptake and milk protein synthesis with AA-CN at 29 DIM seemed to be well correlated with the greater DMI. Therefore, the increased MP supply likely promoted increased mobilization of protein from body reserves in the first week postpartum. Concomitantly, we observed increased arterial NEFA and glycerol concentrations (Galindo et al., 2015), indicating both increased mobilization of body fat and protein in response to increased MP supply in the first week postpartum. Increased plasma NEFA concentrations in the first week postpartum have previously been observed with increased postpartum MP protein supply via abomasal casein infusion (Larsen et al., 2014b) and via soybean meal (Brown and Allen, 2013). Altogether, these observations underline the high mammary demand for AA and nutrients during initiation of lactation. However, the underlying mechanism for the increased mammary milk synthesis remains unclear: the increased synthesis could be caused by either simple fulfillment of a mammary pull of AA or the additional AA may have acted as metabolic signals to stimulate milk protein synthesis (Rius et al., 2010).

CONCLUSIONS

Early postpartum abomasal AA infusion induced a profound increase in milk and milk protein yields with a basis in increased mammary uptake of AA. The PDV metabolism of branched-chain AA increased, whereas that of other EAA seemed to be unaffected when increasing the intestinal AA supply in the early postpartum period. On a net basis, the hepatic removal of group 1 AA increased when the early postpartum AA supply was increased, although the partitioning between anabolic and catabolic metabolic pathways could not be assessed. Thus, the early postpartum protein deficiency persisted and the experiment indicates that increased

early postpartum AA supply may increase mobilization of protein reserves in the first week postpartum.

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