Abomasal protein infusion in postpartum transition dairy cows: Effect on performance and mammary metabolism

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ABSTRACT

The effect of increasing the postpartum metabolizable protein (MP) supply on performance and mammary metabolism was studied using 8 Holstein cows in a complete randomized design. At parturition, cows were assigned to abomasal infusion of water (CTRL) or casein (CAS). Arterial and epigastric venous blood samples were taken 14 d before expected parturition and at 4, 15, and 29 d in milk (DIM). To compensate previously estimated deficiency of essential AA and to avoid oversupply, casein protein infusion was graduated with 696 ± 1 , 490 ± 9 , and 212 ± 10 g/d at 4, 15 and 29 DIM, respectively. Dry matter intake was unaffected by CAS. Compared with CTRL, MP supply was greater at 4 DIM with CAS but did not differ by 29 DIM. Milk yield was greater with CAS ($+7.2 \pm 1.3$ kg/d from 1 to 29 DIM). Milk protein yield was greater with CAS at 4 DIM and averaged $1,664 \pm 39$ g/d compared with $1,212 \pm 86$ g/d for CTRL, but did not differ at 29 DIM ($1,383 \pm 48$ g/d). The ratio of MP total supply to requirement was numerically greater at 4 DIM for CAS compared with CTRL, indicating less postpartum protein deficiency. In contrast, a greater net energy deficiency tended to be induced with CAS, but the greater milk yield allowed a large part of mobilized fat to be secreted in milk. Arterial concentration of total essential AA increased sharply after parturition for CAS compared with slight decreases for CTRL. The patterns of arterial concentrations combined with arterial-mammary venous concentration differences indicated that Lys, Leu, and Tyr were the first-limiting AA at 4 DIM with CTRL. Mammary plasma flow was unaffected by treatment, indicating similar perfusion of mammary tissue. The greater milk yield with CAS was associated with greater mammary uptake of individual essential AA, tendencies to greater uptake of glucose, lactate, and β -hydroxybutyrate, whereas uptakes of

volatile fatty acids were unaffected. Despite similar MP supply by 29 DIM, milk and lactose yields were greater with CAS indicating a persistent response to increased postpartum MP supply. In conclusion, the postpartum MP deficiency can have a substantial negative effect in dairy cows as the major outcome of increasing the postpartum MP supply was increased milk, milk protein, and lactose yield, as well as an enhanced MP balance. Potential positive effects for other body functions than milk synthesis are discussed. Future investigations are needed to delineate how to transfer the effect into practical feeding strategies.

Key words: dairy cow, protein, metabolism, periparturient

INTRODUCTION

The dilemma of the postpartum transition cow having to meet the rapid increase in nutrient requirements for milk synthesis by equivalent increments in nutrient intake has been formulated and emphasized repeatedly (Bell, 1995; Loor, 2010). The consequences of the negative energy balance and associated fat mobilization have received great focus during the past decades (Grummer, 1995; Ingvarsen et al., 2003; Loor, 2010). In contrast, the concomitant negative protein balance has received little attention, even though the extent and potential negative consequences 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, then diminishing and becoming positive during the fourth week postpartum (Bell et al., 2000). Recently, a similar pattern has been observed by relating the release of EAA from total splanchnic tissues to the secretion in milk (Larsen and Kristensen, 2009a; Dalbach et al., 2011). Consequently, during this period, AA are mobilized mainly from skeletal muscles (Gibb et al., 1992) and postpartum involution of smooth muscle in the uterus (Gibb et al., 1992; Andrew et al., 1994). The available protein seem to be partitioned mainly to protein synthesis over glucogenic purposes in the early postpartum period, as EAA did not contribute more to

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liver glucose release on a net basis in the postpartum period (Larsen and Kristensen, 2013).

The negative protein balance in the early postpartum period does further establish low blood concentrations of most EAA relative to the prepartum period (Doepel et al., 2002; Dalbach et al., 2011). The lowered circulating levels of EAA may compromise vital body functions, such as the immune defense system (Li et al., 2007) and proliferation of splanchnic tissues (Gibb et al., 1992). With respect to the mammary gland, it has been observed that the mammary uptake of single EAA can be upregulated when protein supply was low for specific EAA (Bequette et al., 2000; Lapierre et al., 2009). Despite the metabolic flexibility of the mammary gland, it could be speculated that the general lowered circulating levels of EAA in the postpartum period are limiting mammary EAA uptake and milk protein synthesis.

The overall hypothesis of the present study was that the protein deficiency in the postpartum period limits animal performance; therefore, abomasal infusion of casein to postpartum transition cows will (1) increase blood EAA concentration, (2) increase mammary uptake of EAA, and (3) increase milk protein yield. The aim of the study was to investigate the effects of alleviating the protein deficiency in postpartum transition dairy cows on milk production, mammary metabolism, and mobilization of body reserves.

MATERIALS AND METHODS

The present experiment complied with Danish Ministry of Justice Law no. 382 (June 10, 1987), Act no. 726 (September 9, 1993), concerning experiments with animals and care of experimental animals.

Animals, Diets, and Experimental Design

Eight Holstein cows entering their second lactation were used in a complete randomized design with repeated measurements. Sampling days of blood were designated to prepartum (-14.4 ± 3.6 d) and to 4.0 ± 0.0 , 14.9 ± 0.8 , and 29.3 ± 1.0 DIM. At the day of parturition (designated as 1 DIM), cows were randomly assigned to 1 of 2 treatments: continuous abomasal infusion of water (**CTRL**) or casein (**CAS**). Abomasal infusion of casein protein (containing 91.0% CP as fed) was planned to supply 360 g/d at 1 DIM, 720 g/d at 2 DIM, followed by daily reductions of 19.5 g/d ending at 194 g/d at 29 DIM. The amount of casein infused was planned to supply the estimated deficient EAA at 4 and 29 DIM by Dalbach et al. (2011) and avoiding great oversupplies. The obtained casein protein infusion was initiated 6.0 ± 1.7 h ($\pm$ SD) after parturition

and averaged 696 ± 1 , 490 ± 9 , and 212 ± 10 g/d at the 4, 15, and 29 DIM sampling days, respectively. An abomasal infusion device was placed in the abomasum via the ruminal cannula at the day of parturition, as previously described (Larsen and Kristensen, 2009b). The casein infusate was prepared daily as a 7.9% solution of Na-caseinate (Miprodan 30; Arla Foods Ingredients a.m.b.a., Viby, Denmark) using 50 to 55°C warm tap water and a mixer wagon for calf milk replacer. Infusates were infused using silicone tubing (3 mm i.d., 6 mm o.d.; Ole Dich Instrumentmakers, Hvidovre, Denmark) and peristaltic pumps (Type 115/G42 with L-channels; Ole Dich Instrumentmakers).

All cows were offered the same prepartum and the same postpartum diets (Table 1) composed to fulfill the Nordic recommendation for nutrient allowances (Volden, 2011). The protein level in the basal postpartum diet was chosen to parallel feeding regimens using 1 TMR for all lactating cows. The prepartum feed intake was restricted to 10 kg of DM/d; postpartum, cows were fed ad libitum (10% orts). Diets were offered as a TMR and fed in equally sized meals at 0830, 1630, and 0030 h. Orts were weighed and removed at 0800 h. Cows were milked at 0500, 1300, and 2100 h.

Ruminal cannulas (#1C; Bar Diamond, Parma, ID) were implanted 4 wk before dry off, and permanent indwelling catheters in the intercostal artery were implanted 4 to 6 wk before expected parturition, as previously described (Larsen and Kristensen, 2009b). On sampling days, a temporary catheter was placed in the right epigastric vein. Cows were dried off at 8 to 9 wk before expected parturition allowing at least 8 wk of effective dry period. Cows were housed in tiestalls bedded with rubber mats and wood shavings and were moved to a straw-bedded calving pen at signs of parturition. The expelled placenta was removed from the pen as soon as possible after parturition to prevent a protein boost from ingestion of the placenta.

Experimental Sampling and Data Collection

Feed intake and milk yield were recorded daily. Diets were sampled 2 times per week for DM determination, and the dried samples were pooled within diet and week for later chemical analyses. Milk yield was recorded using continuous flow sampling (TruTest HI, Tru-Test Scandinavia, Præstø, Denmark) and milk was sampled on blood sampling days at each milking from the continuous flow sample. Feces were sampled mornings and afternoons of the day before and at the 15 and 29 DIM sampling days. Fecal samples were pooled within cow and sampling day and stored at -20°C until analysis. Cows were weighed before the afternoon feeding of each sampling day.

Table 1. Composition of the total mixed diets fed pre- and postpartum

Item, g/kg of DM (unless otherwise noted)	Prepartum	Postpartum
Ingredient		
Corn silage	450 ¹	350 ²
Grass-clover silage ³	200	200
Barley straw, chopped	120	
Wheat grain, ground	90	
Wheat grain, NaOH treated		200
Soybean meal, 54% CP	100	81
Rapeseed meal		81
Molasses, sugar cane	13	
Sugar beet pulp, dried		60
Vegetable fat ⁴	10	10
CaCO ₃		5.2
NaCl	5	1.3
Mineral-vitamin premix	12 ⁵	7.5 ⁶
E-vitamin premix ⁷		1.5
Ti(IV)O ₂		2.5
Nutrient		
DM, g/kg	425	522
Ash	60	70
CP	141	159
Starch	202	236
NDF ⁸	384	299
Metabolizable protein ⁸	77	97
Metabolizable Lys, % of MP ⁸	6.86	6.64
Metabolizable Met, % of MP ⁸	2.22	2.20
NE _L , MJ/kg of DM ⁸	6.69	6.77

¹Chemical analysis: DM, 29.0%; ash, 3.1% of DM; CP, 9.1% of DM; NDF, 40.2% of DM; starch, 30.9% of DM; in vitro digestible OM, 75.1% of DM.

²Chemical analysis: DM, 33.0%; ash, 3.0% of DM; CP, 9.1% of DM; NDF, 38.2% of DM; starch, 33.3% of DM; in vitro digestible OM, 76.6% of DM.

³Chemical analysis: DM, 47.1%; ash, 8.8% of DM; CP, 13.6% of DM; NDF, 43.6% of DM; sugars, 8.4% of DM; in vitro digestible OM, 72.2% of DM.

⁴Palm fatty acid distillate (PFAD, Scanfedt A/S, Fredericia, Denmark).

⁵Premix dry (Gold D-alfa org. Se; Vitfoss, Gråsten, Denmark) containing (per kilogram): 20 g of Ca, 40 g of P, 192 g of Mg, 37 g of Na, 83 g of S, 1,091 kIU of vitamin A, 181.8 kIU of vitamin D, 12,166 mg of RRR- α -tocopherol, 5,455 mg of Mn, 1,455 mg of Cu, 35 mg of Co, 7,270 mg of Zn, 227 mg of I, 18 mg of Se, and 18 mg of organic Se.

⁶Premix lactation (VM 2; Vitfoss) containing (per kilogram): 160 g of Ca, 5 g of P, 65 g of Mg, 90 g of Na, 2 g of S, 600 kIU of vitamin A, 190 kIU of vitamin D, 3,640 mg of α -tocopherol, 4,000 mg of Mn, 1,500 mg of Cu, 25 mg of Co, 4,500 mg of Zn, 225 mg of I, and 50 mg of Se.

⁷E-vitamin premix (Suplex E-50000/K/selen; Vitfoss) containing (per kilogram): 172 g of Ca, 3 g of P, 45,500 mg of α -tocopherol, 2,300 mg of vitamin K₃, 1,766 mg vitamin B₃, and 100 mg of Se.

⁸Calculated from ingredient analysis using the non-additive feed evaluation system NorFor (Volden, 2011).

On each sampling day, 4 bihourly sets of arterial and mammary venous blood were obtained starting 30 min before feeding at 0830 h. Before collecting the main samples, whole blood samples were collected for immediate blood gas and oximetry measurements using heparinized 2-mL gas syringes (PICO50, Radiometer A/S, Copenhagen, Denmark). Main blood samples were collected using 10- or 20-mL disposable syringes and were immediately stabilized in either heparin

(#455051; Greiner Bio-One GmbH, Kremsmünster, Austria) or K₃ EDTA Vacuette tubes (#455036; Greiner Bio-One GmbH) and placed on crushed ice. Plasma was harvested by centrifugation at 3,000 $\times g$ for 20 min at 4°C and stored at -20°C until analysis.

Analytical Procedures

Feed samples were analyzed for DM, ash, CP, NDF, and starch as described by Kristensen et al. (2007). Dry matter and starch concentrations in feces were determined as described for feed samples. The TiO₂ contents in the postpartum TMR and feces were determined according to (Myers et al., 2004). Milk samples were analyzed for fat, CP, and lactose monohydrate by infrared spectrometry using a MilkoScan 4000 (Foss Electric, Hillerød, Denmark).

All analyses were performed on individual blood or plasma samples. Hematocrit was determined immediately after sampling in heparin-stabilized arterial samples by centrifugation in capillary tubes at 13,000 $\times g$ for 6 min at ambient temperature. Blood pH, blood gasses, and oximetry variables were measured using an ABL700 Blood Gas Analyzer (Radiometer A/S, Copenhagen, Denmark). Heparinized plasma samples were analyzed for glucose and L-lactate using D-glucose oxidase and L-lactate oxidase, respectively (YSI 7100, YSI Inc., Yellow Springs, OH), and for BHBA, NEFA (K₃ EDTA plasma), and ammonia using a Cobas Mira autoanalyzer (Triolab A/S, Brøndby, Denmark) and enzymatic kits based on D-3-hydroxybutyrate dehydrogenase, acyl CoA synthetase/acyl CoA oxidase, and glutamate dehydrogenase, respectively (Ranbut 1008, FA 115, and AM 1015, respectively, Randox Laboratories Ltd., Crumlin, UK). Plasma insulin and IGF-1 were determined by time-resolved fluoroimmunoassay according to Løvendahl and Purup (2002) and Frystyk et al. (1995), respectively. Intra- and interassay coefficients of variation were 3.3 and 3.0%, respectively, for insulin, and 3.3 and 3.2%, respectively, for IGF-1.

Heparinized plasma samples were analyzed for AA, 3-methyl-His (**3MH**), hydroxy-Pro (**Hyp**), and urea by GC-MS using the isotope dilution method (Calder et al., 1999). A working AA standard solution was prepared from a commercial AA mixture (AAS18, Sigma-Aldrich Denmark A/S, Brøndby, Denmark) with added Gln (L-Gln 99%, final concentration 400 μM , Acros, Geel, Belgium), 3MH (N γ -methyl-L-histidine, final concentration 200 μM , Sigma-Aldrich Denmark A/S), Hyp (hydroxy-L-proline, final concentration 200 μM , Sigma-Aldrich Denmark A/S), and urea (final concentration 7,500 μM , Merck, Darmstadt, Germany). The internal standard was made from a (U-¹³C/U-¹⁵N) cell-free AA mixture (CNLM-6696-1; Cambridge Isotope Labora-

tories Inc., Andover, MA) with added τ -[D₃]methyl-L-His (DLM-2949; Cambridge Isotope Laboratories, Inc.), hydroxy-[2,5,5-D₃]L-Pro (D-7186; CDN Isotopes, Pointe-Claire, Quebec, Canada), and [¹⁵N₂] urea (no. 316830; Campro Scientific GmbH, Berlin, Germany). Ions used for detection of 3MH, Hyp, and urea were 340, 314, and 231 m/z, respectively. Concentrations of VFA in heparinized plasma samples were determined by GC according to Kristensen (2000) coupled with a mass spectrometer as detector.

Calculations and Statistical Procedures

The MP supply from infused casein was calculated using a small intestinal digestibility of 94.6% (Chaudhry and Webster, 1993; Rutherford and Moughan, 1998). The utilization of NE_L and MP was estimated as the ratio between total supply (including infused Na-caseinate) and requirement of NE_L and MP calculated using the nonadditive Nordic feed evaluation system NorFor (Volden, 2011). The total-tract apparent digestibility of DM, CP, and starch was calculated for 15 and 29 DIM using TiO₂ as an indigestible marker and average nutrient intake of the 2 d preceding the respective sampling day. Average milk composition was calculated taking differences in milk yield at morning, afternoon, and evening milkings into account. Energy-corrected milk yield was calculated taking lactose monohydrate concentration into account (Sjaunja et al., 1991). Each postpartum change in BW was calculated relative to the prepartum observation.

Whole blood concentrations of VFA were estimated from plasma concentrations by correcting for a 45% dilution space in erythrocytes (Kristensen, 2000). The ammonia concentration in whole blood was set equal to the plasma concentration, whereas the whole blood concentration of urea N was obtained by correcting for a urea dilution space in erythrocytes of 80% (Røjen et al., 2011). Mammary blood plasma flow was calculated using the Fick's principle with Phe and Tyr as markers, accounting for 3.37% blood-borne proteins and 5.0% NPN content in milk protein (Lapierre et al., 2012). Mammary whole blood flow was calculated by relating plasma flow to the simultaneous arterial hematocrit value. The mammary net flux of nutrients was calculated as the arterial-venous concentration difference multiplied by the appropriate mammary plasma or blood flow. The milk secretion of Phe and Tyr, as well as other AA, were calculated using an average AA composition of milk protein (Lapierre et al., 2012).

Data were subjected to ANOVA using the MIXED procedure in SAS (ver. 9.2, release TS1M0, SAS Institute Inc., Cary, NC). Postpartum observations were

analyzed using a model including the fixed effects of treatment (**Trt**), DIM, sampling time relative to morning feeding and the possible interactions; DIM within cow and time relative to feeding within cow and DIM were considered as repeated measurements using compound symmetry and autoregressive order 1 covariance structures, respectively. For variables with 1 observation per day (daily or sampling days), a reduced model was used including Trt, DIM, and the Trt × DIM interaction; DIM within cow was considered as a repeated measure using the autoregressive order 1 covariance structure. The denominator degrees of freedom were calculated using the Kenward-Roger method. The LSM ± SEM are presented as missing observations were present: udder edema prevented one epigastric sampling with CTRL at 4 DIM, and a mastitis incidence with CTRL at 4 DIM; thus, $n = 2$ for mammary data with CTRL at 4 DIM. Treatment means within DIM were separated using protected Fisher's least significant difference test. The prepartum observations were subsequently included to test the prepartum to 4 DIM difference × treatment interaction ($P_{\text{Trans} \times \text{trt}}$) and for the general prepartum to 4 DIM difference (P_{Trans}) using Fisher's least significant difference tests. Significance was declared at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

RESULTS

No calving problems and illnesses other than mastitis were observed (Table 2). Two incidences of mastitis were observed and cows responded quickly to veterinary treatment in both cases. One mastitis incidence was observed the evening before the 4 DIM sampling with a cow on CTRL; thus, mammary uptake of nutrients was not used for this observation.

Feed Intake, Digestibility, Milk Production, and BW

The voluntary DMI was not affected by treatment ($P = 0.35$; Figure 1a) and increased ($P < 0.01$) as lactation progressed with both treatments. Over the entire period, the profiled casein infusion gave higher and more constant postpartum MP supply with CAS com-

Table 2. Description of cows within treatment group (mean ± SD)¹

Item	CTRL	CAS
BW at dry off, kg	562 ± 70	581 ± 50
BCS at dry off, 1–5 scale	2.9 ± 0.1	3.0 ± 0.2
BW of born calves, kg	40.5 ± 3.5	39.3 ± 6.0
Twin births, no.	0	0

¹Continuous abomasal infusion of water (CTRL) or Na-caseinate (CAS) from 1 to 29 DIM.

pared with CTRL (2.13 ± 0.02 vs. 1.81 ± 0.05 kg/d; $P_{\text{Trt} \times \text{DIM}} = 0.01$; Figure 1b). The apparent total-tract digestibility of DM, CP, and starch were not affected by treatment ($P = 0.25$ to $P = 0.59$; Table 3).

Through the experimental period, milk yield was constantly greater ($+7.2 \pm 1.3$ kg/d; $P < 0.01$; Figure 1c) for CAS compared with CTRL, and increased as lactation progressed with both treatments ($P < 0.01$). Milk protein yield with CAS averaged $1,664 \pm 39$ g/d at 4 DIM compared with $1,212 \pm 86$ g/d for CTRL, whereas milk protein yield did not differ at 29 DIM ($P_{\text{Trt} \times \text{DIM}} = 0.03$; Table 3). Milk protein concentration decreased more rapidly postpartum with CAS compared with CTRL ($P_{\text{Trt} \times \text{DIM}} = 0.05$). Treatment neither affected absolute BW nor change in BW ($P = 0.75$ and $P = 0.78$, respectively; Table 3). The utilization of total NE_L tended to be lower with CAS compared with CTRL ($P = 0.08$; Table 3), indicating a greater NE_L deficiency with CAS. Numerically, the utilization of total MP increased faster postpartum for CTRL from below 100% compared with a slower increase for CAS from above 100% ($P_{\text{Trt} \times \text{DIM}} = 0.11$), indicating less postpartum protein deficiency with CAS.

Arterial Concentrations

The arterial concentrations of glucose, lactate, BHBA, insulin, and IGF-1 were not affected by treatment ($P = 0.25$ to $P = 0.71$; Table 4). The arterial concentration of NEFA tended to increase more from prepartum to 4 DIM for CAS compared with CTRL ($P_{\text{Trans} \times \text{trt}} = 0.07$), but were not affected by treatment at 15 and 29 DIM ($P_{\text{Trt} \times \text{DIM}} = 0.06$). The arterial concentrations of 3MH and Hyp increased sharply from prepartum to 4 DIM ($P < 0.01$), but were unaffected by treatment ($P_{\text{Trans} \times \text{trt}} = 0.69$ and $P_{\text{Trans} \times \text{trt}} = 0.45$, respectively); postpartum, the concentration of 3MH remained unaffected by treatment ($P = 0.92$), whereas the concentration of Hyp tended ($P = 0.08$) to be lower for CAS compared with CTRL.

The arterial concentration of total EAA increased sharply after parturition for CAS compared with CTRL ($P_{\text{Trans} \times \text{trt}} < 0.01$). Arterial concentrations of Ile, Leu, Lys, and Val either decreased or did not change from prepartum to 4 DIM for CTRL, but increased with CAS ($0.01 \leq P_{\text{Trans} \times \text{trt}} \leq 0.05$; Table 4). For His, Met, Phe, Thr, and Trp, no effect of treatment was observed on prepartum to 4 DIM difference ($P \geq 0.18$). Postpartum, concentrations of Leu, Lys, and Val increased with CTRL as lactation progressed compared with steady or decreasing concentrations for CAS ($0.01 \leq P_{\text{Trt} \times \text{DIM}} \leq 0.11$). Arterial concentrations of His tended ($P = 0.06$) to be greater with CAS compared with CTRL. The concentration of Phe tended to be greater at 4 DIM

Figure 1. Voluntary DMI (a), metabolizable protein (MP) supply (b), and milk yield (c) in postpartum transition Holstein cows subjected to continuous abomasal infusion of either water (○; CTRL) or casein (□; CAS) initiated at the day of parturition. Each data point is the mean of 4 observations $\pm$ SE. Asterisks (*) signify treatment means to differ within DIM ($P \leq 0.05$). The DMI was not affected by treatment ($P = 0.35$; a) and increased ($P < 0.01$) as lactation progressed with both treatments. The profiling of casein infusion resulted in a high and more constant supply of MP from 2 to 29 DIM for CAS as compared with CTRL (2.13 ± 0.02 vs. 1.81 ± 0.05 kg/d; $P_{\text{treatment} \times \text{DIM}} = 0.01$; b). Milk yield was greater ($+7.2 \pm 1.3$ kg/d; $P < 0.01$; c) with CAS compared with CTRL, and increased ($P < 0.01$) as lactation progressed with both treatments.

Table 3. Intake, apparent total-tract digestibility, milk yield, milk composition, BW, and utilization of energy and protein¹

Item	Prepartum		4 DIM		15 DIM		29 DIM		SEM ²	P-value, 4 to 29 DIM ³			P-value, prepartum to 4 DIM	
	CTRL	CAS	CTRL	CAS	CTRL	CAS	CTRL	CAS		Trt	DIM	Trt × DIM	Trans × Trt ⁴	Trans ⁵
Intake														
DM diet, kg/d	10.1	10.1	14.3	14.9	18.8	17.8	22.1	19.9	1.1	0.50	<0.01	0.32	0.73	<0.01
CP diet, kg/d	1.43	1.46	2.26	2.35	2.97	2.83	3.52	3.17	0.17	0.51	<0.01	0.36	0.83	<0.01
Starch, kg/d	2.02	2.11	3.36	3.44	4.62	4.39	5.09	4.82	0.26	0.45	<0.01	0.43	0.98	<0.01
NE _L , MJ/d	67	67	100	111	128	127	148	137	6.5	0.95	<0.01	0.17	0.31	<0.01
MP, g/d	775	775	1,250	1,964**	1,795	2,114	2,226	2,115	133	0.09	<0.01	0.01	<0.01	<0.01
Total-tract apparent digestibility, %														
DM	—	—	—	—	71.6	73.8	70.4	72.3	1.2	0.25	0.05	0.80	—	—
CP	—	—	—	—	64.3	62.1	61.6	61.2	1.3	0.42	0.16	0.42	—	—
Starch	—	—	—	—	95.8	97.0	95.9	96.8	1.4	0.59	0.96	0.80	—	—
Milk yield														
Milk, kg/d	—	—	27.4	36.9**	37.6	46.8**	41.0	48.9**	1.6	<0.01	<0.01	0.82	—	—
ECM, kg/d	—	—	32.0	43.5**	38.5	46.3*	40.0	43.2	2.1	0.02	0.03	0.12	—	—
Fat, g/d	—	—	1,345	1,814**	1,523	1,843†	1,616	1,630	115	0.07	0.46	0.13	—	—
Protein, g/d	—	—	1,212	1,664**	1,374	1,586*	1,319	1,447	67	0.01	0.11	0.03	—	—
Lactose, g/d	—	—	1,213	1,669**	1,807	2,254**	1,964	2,369**	78	<0.01	<0.01	0.93	—	—
Milk composition, g/kg														
Fat	—	—	48	49	41	39	39	33	2.3	0.29	<0.01	0.38	—	—
Protein	—	—	44	45	37	34†	32	30†	1.0	0.24	<0.01	0.05	—	—
Lactose	—	—	44	45	48	48	48	49	0.5	0.20	<0.01	0.76	—	—
Urea, mM	—	—	3.2	4.8**	3.0	4.3**	3.1	4.0*	0.3	<0.01	0.26	0.55	—	—
BW, kg	614	631	585	603	562	576	562	563	25	0.75	<0.01	0.60	0.96	0.10
BW change, kg	—	—	−29	−28	−52	−55	−52	−68	14	0.78	<0.01	0.57	—	—
Utilization, ⁶ %														
NE _L	132	130	76	65	83	71†	93	80†	4.9	0.08	0.01	0.97	0.32	<0.01
MP	198	199	97	113	121	125	151	135	7.6	0.90	<0.01	0.11	0.27	<0.01

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or Na-caseinate (CAS) from 1 to 29 DIM; casein protein infusion averaged 696 ± 1, 490 ± 9, and 212 ± 10 g/d at 4, 15, and 29 DIM, respectively.

²n = 4.

³Fixed effects when prepartum observations are excluded from data set.

⁴Fisher's LSD test for treatment effect on difference between prepartum and 4 DIM.

⁵Fisher's LSD test for prepartum to differ from 4 DIM.

⁶Utilization (intake:requirement) calculated including infused Na-caseinate.

** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.10$: symbols signify difference between LSM within DIM and rows.

Table 4. Arterial variables (μM unless otherwise noted)¹

Item	Prepartum		4 DIM		15 DIM		29 DIM		SEM ²	<i>P</i> -value, 4 to 29 DIM ³			<i>P</i> -value, prepartum to 4 DIM	
	CTRL	CAS	CTRL	CAS	CTRL	CAS	CTRL	CAS		Trt	DIM	Trt × DIM	Trans × Trt ⁴	Trans ⁵
Blood plasma														
Glucose, mM	3.57	3.72	3.07	2.97	3.07	2.99	3.31	3.13	0.17	0.54	0.23	0.92	0.33	<0.01
L-Lactate, mM	0.53	0.50	0.48	0.35	0.28	0.24	0.28	0.26	0.05	0.25	0.01	0.45	0.23	0.03
NEFA	154	173	376	572**	306	321	153	166	45	0.12	<0.01	0.06	0.07	<0.01
BHBA	791	723	742	1,344	731	1,202	669	1,168	379	0.28	0.89	0.97	0.22	0.29
Insulin, pM	62	53	10	16	21	18	22	27	5.9	0.71	0.01	0.36	0.08	<0.01
IGF-1, ng/mL	173	187	48	59	70	90	73	84	11	0.36	<0.01	0.79	0.90	<0.01
3-Methyl-His	6.2	7.0	14	14	5.2	5.2	3.7	3.7	1.0	0.92	<0.01	0.99	0.69	<0.01
Hydroxy-Pro	14	14	21	18	15	13	14	12	1.2	0.08	<0.01	0.90	0.45	<0.01
EAA	630	674	583	1,026**	833	985	772	900	96	0.08	0.27	0.04	<0.01	0.02
His	48	61	48	58	46	54	45	55	4.1	0.06	0.62	0.97	0.68	0.60
Ile	91	104	92	175	121	154	107	143	27	0.20	0.68	0.19	0.05	0.05
Leu	93	98	75	183**	115	158	98	129	24	0.08	0.38	0.07	0.01	0.06
Lys	65	64	49	82**	72	77	70	78	5.4	0.06	0.01	<0.01	<0.01	0.72
Met	19	22	21	24	23	21	23	22	1.8	0.91	0.92	0.19	0.79	0.16
Phe	44	45	43	50	51	50	46	48	3.6	0.63	0.07	0.09	0.18	0.38
Thr	76	82	78	93	116	102	132	121	17	0.86	0.01	0.41	0.69	0.54
Trp	32	29	28	30	43	39	41	41	1.8	0.70	<0.01	0.10	0.21	0.41
Val	161	168	151	332**	246	314	209	264	44	0.09	0.32	0.11	0.01	0.02
NEAA	1,070	1,195	1,233	1,328	1,371	1,443	1,292	1,272	66	0.53	0.04	0.50	0.75	<0.01
Ala	184	171	178	143	214	184	234	199	21	0.24	<0.01	0.98	0.42	0.22
Asn	32	38	35	43	52	52	54	55	2.3	0.35	<0.01	0.10	0.65	0.04
Asp	3.9	4.0	2.3	3.0	3.2	3.4	3.1	3.7	0.6	0.55	0.02	0.63	0.35	<0.01
Cys	111	98	82	95	120	109	125	109	7.5	0.68	<0.01	<0.01	<0.01	<0.01
Gln	292	381	202	248	183	239	184	228	20	0.10	0.17	0.81	0.23	<0.01
Glu	69	68	41	40	46	43	41	43	4.0	0.91	0.16	0.43	0.96	<0.01
Gly	208	253	476	480	486	514	413	393	32	0.91	0.01	0.66	0.40	<0.01
Pro	60	60	63	127**	89	122**	89	98	5.7	<0.01	0.03	<0.01	<0.01	<0.01
Ser	69	82	127	102	130	114	106	96	16	0.32	0.33	0.85	0.15	0.01
Tyr	41	42	29	46**	47	47	43	43	3.8	0.24	0.01	0.01	<0.01	0.27
Total AA	1,700	1,878	1,818	2,355**	2,204	2,424	2,064	2,173	99	0.07	<0.01	<0.01	<0.01	<0.01
Whole blood														
Hematocrit, %	28.7	29.9	31.6	31.3	28.3	28.0	27.1	26.1	0.66	0.56	<0.01	0.29	0.03	<0.01
pH	7.45	7.45	7.45	7.45	7.42	7.43	7.44	7.43	0.01	0.91	0.04	0.40	0.57	0.83
O ₂ , mM	6.0	6.4	6.6	6.7	5.9	6.1	5.7	5.7	0.14	0.61	<0.01	0.07	0.05	<0.01
CO ₂ , mM	24.2	23.5	27.1	26.2	26.5	25.8	26.3	26.2	0.67	0.52	0.52	0.66	0.88	<0.01
Ammonia	91	86	91	81	82	78	77	72	5.7	0.43	<0.01	0.38	0.52	0.46
Urea, mM	3.7	3.6	3.3	4.7**	3.1	4.2*	3.3	4.0	0.3	0.01	0.37	0.49	0.01	0.21
Acetate	1,564	1,364	1,288	1,382	1,569	1,662	1,633	1,786	164	0.62	<0.01	0.86	0.07	0.10
Propionate	25	17	41	39	59	52	48	52	6.1	0.83	0.01	0.44	0.44	<0.01
Butyrate	18	14	32	33	50	42	35	44†	3.6	0.86	<0.01	0.02	0.38	<0.01
Isobutyrate	3.1	2.1	4.6	4.1	5.4	4.0	4.7	4.6	1.2	0.67	0.82	0.50	0.61	<0.01
Valerate	0.4	0.4	0.7	0.7	1.0	0.6	1.0	0.5	0.3	0.41	0.93	0.44	0.89	0.17
Isovalerate	3.7	2.8	4.3	4.2	5.1	4.4	4.5	4.8	0.5	0.77	0.20	0.25	0.13	<0.01
Caproate	0.5	0.3	0.3	1.0	0.3	1.3	0.8	1.4	0.3	0.11	0.13	0.66	0.05	0.18

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or Na-caseinate (CAS) from 1 to 29 DIM; casein protein infusion averaged 696 ± 1, 490 ± 9, and 212 ± 10 g/d at 4, 15, and 29 DIM, respectively.

²n = 4.

³Fixed effects when prepartum observations are excluded from data set.

⁴Fisher's LSD test for treatment effect on difference between prepartum and 4 DIM.

⁵Fisher's LSD test for prepartum to differ from 4 DIM.

***P* ≤ 0.01, **P* ≤ 0.05, †*P* ≤ 0.10: symbols signify difference between LSM within DIM and rows.

for CAS compared with CTRL and did not differ at 29 DIM ($P_{\text{Trt} \times \text{DIM}} = 0.09$). Arterial concentrations of Met and Thr were generally unaffected by treatment ($P \geq 0.86$). Overall, concentrations of total nonessential AA were not affected by treatment ($P = 0.50$) but increased with DIM ($P = 0.05$). Individually, arterial concentrations of Cys, Pro, and Tyr either decreased or did not change from prepartum to 4 DIM for CTRL compared with increased concentrations with CAS ($P_{\text{Trans} \times \text{trt}} < 0.01$; Table 4); postpartum, concentrations increased with CTRL as lactation progressed compared with steady or decreasing levels for CAS ($P_{\text{Trt} \times \text{DIM}} \leq 0.01$).

The arterial concentrations of urea increased more from prepartum to 4 DIM for CAS compared with CTRL ($P_{\text{Trans} \times \text{trt}} = 0.01$); postpartum, arterial urea concentrations were greater for CAS compared with CTRL ($P = 0.01$). Concentrations of acetate and propionate in arterial plasma were unaffected by treatment ($0.62 \leq P \leq 0.83$; Table 4) and increased as lactation progressed ($P \leq 0.01$). The arterial concentration of butyrate was greater at 29 DIM for CAS, whereas concentrations did not differ at 4 and 15 DIM ($P_{\text{Trt} \times \text{DIM}} = 0.02$).

Mammary Arterial-Venous Concentration Differences

The mammary arterial-venous (A-V) concentration difference of glucose tended to increase more from prepartum to 4 DIM with CAS compared with CTRL ($P_{\text{Trans} \times \text{trt}} = 0.08$; Table 5). Postpartum, the mammary A-V concentration difference of glucose was unaffected ($P = 0.47$) by treatment, but increased with progressing lactation ($P < 0.01$). For lactate, the mammary A-V concentration difference was negative prepartum, indicating a net release of lactate by the mammary gland. The mammary A-V concentration difference of lactate increased from negative for CTRL and from zero for CAS at 4 DIM to positive and not differing between treatments at 29 DIM ($P_{\text{Trt} \times \text{DIM}} = 0.01$). The mammary A-V concentration difference of BHBA tended to be greater with CAS compared with CTRL ($P = 0.09$) and increasing with DIM for both treatments ($P < 0.01$).

The mammary A-V concentration differences of Leu, Lys, and Tyr increased more from prepartum to 4 DIM for CAS compared with CTRL ($0.01 \leq P_{\text{Trans} \times \text{trt}} \leq 0.09$; Table 5). The increase in mammary A-V concentration differences of His, Ile, Met, Phe, Thr, Trp, and Val from prepartum to 4 DIM were unaffected by treatment ($0.14 \leq P_{\text{Trans} \times \text{trt}} \leq 0.82$). Postpartum, mammary A-V concentration differences of EAA other than His and Val were greater at 4 DIM for CAS compared with CTRL, but did not differ by 29 DIM ($0.01 \leq P_{\text{Trt} \times \text{DIM}} \leq 0.12$). The mammary A-V concentration

difference of His tended to be greater for CAS ($P_{\text{Trt}} = 0.10$) compared with CTRL, whereas mammary A-V concentration difference of Val was unaffected by treatment ($P_{\text{Trt}} = 0.25$), yet increasing with the progressing lactation ($P_{\text{DIM}} < 0.01$). For the nonessential AA, mammary A-V concentration differences of Asn, Cys, Pro, and Tyr were greater at 4 DIM for CAS compared with CTRL, but did not differ by 29 DIM ($P_{\text{Trt} \times \text{DIM}} \leq 0.03$).

The mammary A-V concentration differences of acetate, propionate, and butyrate were unaffected by initiation of lactation ($0.13 \leq P_{\text{Trans}} \leq 0.73$; Table 5). Postpartum, mammary A-V concentration differences of acetate, propionate, and butyrate were unaffected by treatment ($P_{\text{Trt}} \geq 0.63$), but increased with the progressing lactation for both treatments ($0.01 \leq P_{\text{DIM}} \leq 0.07$).

Net Mammary Uptake of Nutrients

The absolute mammary plasma flow was not affected by CAS infusion or DIM ($P \geq 0.24$; Table 6), but when expressed relative to milk yield it was greater with CTRL at 4 DIM compared with CAS and did not differ between treatments by 29 DIM ($P_{\text{Trt} \times \text{DIM}} = 0.11$). The mammary net uptake of glucose and BHBA tended to be greater for CAS compared with CTRL from 4 through to 29 DIM ($P = 0.10$ and $P = 0.08$, respectively). A net release of lactate (negative net flux) by the mammary gland was observed at 4 DIM for CTRL compared with a net uptake for CAS, but by 29 DIM; net lactate uptake was observed with both treatments ($P_{\text{Trt} \times \text{DIM}} = 0.06$).

Mammary uptake of total EAA was greater for CAS compared with CTRL ($P < 0.01$) and increased with increasing DIM ($P = 0.01$; Table 6). Mammary uptake of each individual EAA was or tended to be greater for CAS compared with CTRL ($0.01 < P \leq 0.11$). Mammary uptake of total nonessential AA tended to increase ($P = 0.06$) with CAS infusion and decreased ($P = 0.01$) with DIM. The mammary uptake of acetate, propionate, and butyrate were unaffected by treatment ($0.44 \leq P \leq 0.90$). Mammary uptake of O_2 and release of CO_2 were numerically larger ($P \leq 0.18$) for CAS than for CTRL.

The mammary net uptake-to-milk output ratio (U:O) of His increased numerically to be above unity as lactation progressed for CAS as compared with U:O ratios not different from unity for CTRL ($P_{\text{Trt} \times \text{DIM}} = 0.13$; Table 7). For Met and Trp, the U:O ratios were not different from unity and were unaffected by treatment ($P = 0.72$ and $P = 0.71$, respectively). The U:O ratio for Lys was greater for CAS compared with CTRL ($P = 0.01$) and increased numerically with the progressing lactation for both treatments ($P = 0.14$).

Table 5. Arterial – mammary venous metabolite concentration differences (μM unless otherwise noted)¹

Item	Prepartum		4 DIM		15 DIM		29 DIM		SEM ²	P-value: 4 to 29 DIM ³			P-value: pre to 4 DIM	
	CTRL	CAS	CTRL	CAS	CTRL	CAS	CTRL	CAS		Trt	DIM	Trt × DIM	Trans × Trt ⁴	Trans ⁵
Blood plasma														
Glucose, mM	0.18	0.18	0.49	0.66	0.82	0.86	0.90	0.90	0.06	0.47	<0.01	0.29	0.08	<0.01
L-Lactate, mM	−0.07	−0.09	−0.07	0.03**	0.04	0.04	0.07	0.06	0.02	0.44	<0.01	0.01	0.12	0.13
BHBA	135	122	149	244†	305	374	328	385	30	0.09	<0.01	0.64	0.09	0.03
EAA	47	70	209	269*	274	303	267	290	15	0.07	0.01	0.26	0.19	<0.01
His	3.7	3.9	11	12	12	15	12	16†	1.4	0.10	0.21	0.92	0.82	<0.01
Ile	6.9	12	30	39*	40	43	41	40	2.4	0.30	0.01	0.04	0.31	<0.01
Leu	11	15	44	58	57	62	57	57	3.6	0.21	0.09	0.11	0.09	<0.01
Lys	13	16	34	50**	48	52	46	51	2.3	0.03	0.01	0.01	<0.01	<0.01
Met	2.6	3.6	10	13†	13	13	13	13	1.0	0.65	0.47	0.07	0.14	<0.01
Phe	2.1	3.3	17	21†	22	22	21	21	1.3	0.48	0.24	0.12	0.17	<0.01
Thr	2.7	4.4	22	29*	29	27	27	26	1.6	0.60	0.41	0.07	0.20	<0.01
Trp	0.6	0.7	3.9	5.3	5.0	5.0	5.5	5.2	0.4	0.46	0.42	0.12	0.38	<0.01
Val	5.0	10	34	44	49	55	49	49	3.2	0.25	<0.01	0.22	0.54	<0.01
NEAA	27	55	188	242	217	234	203	233	18	0.15	0.77	0.36	0.59	<0.01
Ala	0.5	2.3	41	37	41	37	42	41	3.0	0.37	0.63	0.87	0.36	<0.01
Asn	2.3	5.5	13	18*	19	17	19	17	1.2	0.84	0.35	0.01	0.64	<0.01
Asp	1.2	1.2	2.2	2.1	2.6	2.4	2.6	2.7	0.3	0.89	0.02	0.57	0.84	0.01
Cys	1.2	3.5	−0.2	4.8*	5.6	4.4	4.7	3.5	1.2	0.78	0.28	0.03	0.32	0.95
Gln	32	38	49	76	51	59	42	54	11	0.31	0.06	0.26	0.51	0.22
Glu	19	23	24	26	30	27	27	28	2.8	0.82	0.20	0.22	0.76	0.07
Gly	−27	−18	3.2	6.7	4.5	7.4	7.5	7.6	3.3	0.65	0.57	0.73	0.67	<0.01
Pro	−0.6	0.2	18	31**	17	25**	17	20	1.7	0.01	<0.01	0.01	<0.01	<0.01
Ser	−3.0	−4.8	18	28	21	24	25	27	2.8	0.28	0.29	0.19	0.07	<0.01
Tyr	2.4	4.0	15	21**	20	21	21	21	1.2	0.24	0.31	0.03	0.07	<0.01
Total AA	74	125	398	511*	491	537	470	522	31	0.09	0.18	0.30	0.38	<0.01
Whole blood														
O ₂ , mM	2.0	1.8	1.4	1.5	1.6	1.7	1.8	1.8	0.1	0.64	<0.01	0.44	0.23	<0.01
CO ₂ , mM	−2.1	−2.4	−1.8	−1.9	−2.0	−2.2	−2.5	−2.2	0.2	0.97	0.11	0.41	0.70	0.07
Ammonia	−1.5	−0.2	−0.6	2.1	6.6	8.8	8.8	10	1.5	0.12	<0.01	0.91	0.69	0.36
Urea	13	95	−15	−34	−33	−37	−13	−8	14	0.68	0.16	0.64	0.08	0.01
Acetate	666	728	668	739	1,043	980	1,119	1,146	61	0.99	<0.01	0.26	0.93	0.73
Propionate	13	12	15	20	30	23	20	21	3.7	0.64	0.07	0.25	0.41	0.13
Butyrate	8.3	6.8	3.1	7.4	11.8	10.1	9.7	11.1	2.0	0.63	0.03	0.29	0.12	0.22
Isobutyrate	0.6	0.3	0.4	−0.6	−0.4	−0.1	−0.6	−0.4	0.4	0.85	0.68	0.25	0.38	0.17
Valerate	−0.3	−0.04	−0.3	0.3	0.4	0.1	0.6	−0.1	0.3	0.40	0.91	0.10	0.47	0.60
Isovalerate	1.4	0.8	0.8	0.9	0.9	0.3	0.8	0.7	0.3	0.45	0.13	0.14	0.11	0.25
Caproate	0.3	−0.6	−0.4	−1.0	−1.4	−1.2	−1.2	−1.5	0.4	0.69	0.23	0.38	0.73	0.19

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or Na-caseinate (CAS) from 1 to 29 DIM; casein protein infusion averaged 696 ± 1, 490 ± 9, and 212 ± 10 g/d at 4, 15, and 29 DIM, respectively.

²n = 4.

³Fixed effects when prepartum observations are excluded from data set.

⁴Fisher's LSD test for treatment effect on difference between prepartum and 4 DIM.

⁵Fisher's LSD test for prepartum to differ from 4 DIM.

** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.10$: symbols signify difference between LSM within DIM and rows.

Table 6. Mammary blood plasma flow and metabolite net fluxes (mmol/h)¹

Item	4 DIM		15 DIM		29 DIM		SEM ²	<i>P</i> -value		
	CTRL	CAS	CTRL	CAS	CTRL	CAS		Trt	DIM	Trt × DIM
Plasma flow										
L/h	976	958	813	901	774	859	69	0.43	0.24	0.74
L/kg of milk	824	621**	521	463	453	422	33	0.05	<0.01	0.11
Blood plasma fluxes										
Glucose	475	608	672	777	675	770	60	0.10	0.04	0.96
L-Lactate	-53	30**	29	33	45	53	14	0.14	0.03	0.06
BHBA	156	233	247	345	258	337	40	0.08	0.06	0.94
EAA	191	260**	221	277**	200	242*	12	<0.01	0.01	0.50
His	10	12	9.9	13*	9.4	14*	1.0	0.01	0.69	0.47
Ile	27	37*	32	38†	31	34	2.2	0.05	0.15	0.35
Leu	41	55**	46	56*	43	49	2.8	0.01	0.12	0.39
Lys	30	47**	38	47*	34	43**	2.2	<0.01	0.08	0.15
Met	9.5	13**	11	12	10	11	0.6	0.06	0.17	0.17
Phe	16	20**	17	20†	16	18	0.9	0.04	0.04	0.35
Thr	21	27*	23	25	20	22	1.3	0.10	0.01	0.14
Trp	3.7	5.0	4.0	4.5	4.1	4.5	0.3	0.11	0.77	0.41
Val	32	42*	40	50*	37	42	2.6	0.04	<0.01	0.32
NEAA	174	237*	174	212	152	198†	15	0.06	0.01	0.19
Ala	39	35	33	34	32	35	3.3	0.95	0.62	0.62
Asn	12	17**	16	15	14	15	1.0	0.23	0.55	0.03
Asp	2.2	1.9	2.2	2.2	2.0	2.3	0.3	0.90	0.68	0.64
Cys	-0.3	4.5**	4.5	4.0	3.6	3.2	1.0	0.39	0.27	0.05
Gln	43	72	41	53	31	45	9.3	0.18	0.07	0.49
Glu	23	25	24	24	21	24	2.7	0.72	0.45	0.57
Gly	2.5	6.2	3.7	6.8	5.7	7.3	3.0	0.47	0.71	0.90
Pro	18	30**	14	22**	13	17	1.5	<0.01	<0.01	0.02
Ser	17	26	17	22	19	23	2.8	0.13	0.45	0.57
Tyr	14	20**	16	19*	16	18	0.8	0.02	0.37	0.02
Total AA	365	497**	395	488*	352	441*	25	0.02	0.01	0.39
Whole blood fluxes										
O ₂	1,826	2,049	1,869	2,159	1,895	2,034	146	0.17	0.85	0.85
CO ₂	-2,424	-2,660	-2,310	-2,812	-2,626	-2,582	219	0.18	0.97	0.56
Ammonia	-0.9	3.0	7.4	11.1	9.1	11.5	1.5	0.01	<0.01	0.91
Urea	-14	-51	-35	-49	-10	-7	14	0.43	0.02	0.39
Acetate	941	1,016	1,205	1,226	1,180	1,320	119	0.54	0.13	0.83
Propionate	21	27	37	28	21	24	5.9	0.90	0.15	0.39
Butyrate	3.7	10	14	13	10	13	2.7	0.44	0.17	0.37
Isobutyrate	0.5	-1.0	-0.6	-0.1	-0.7	-0.5	0.5	0.71	0.91	0.31
Valerate	-0.6	0.4	0.6	0.1	0.7	-0.1	0.4	0.46	0.87	0.16
Isovalerate	1.3	1.3	1.2	0.4	0.9	0.9	0.3	0.47	0.06	0.08
Caproate	-0.6	-1.4	-1.7	-1.4	-1.3	-1.8	0.6	0.64	0.70	0.61

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or Na-caseinate (CAS) from 1 to 29 DIM; casein protein infusion averaged 696 ± 1, 490 ± 9, and 212 ± 10 g/d at 4, 15, and 29 DIM, respectively.

²n = 4.

***P* ≤ 0.01, **P* ≤ 0.05, †*P* ≤ 0.10: symbols signify difference between LSM within DIM and rows.

from unity at 4 DIM with CTRL, but from above unity for CAS. For Ile, Leu, and Val, the U:O ratios were greater than unity at all times and increased with progressing lactation for Val (*P* < 0.01) but did not change for Ile and Leu (*P* ≥ 0.22).

DISCUSSION

The overall hypothesis of the present study was that the postpartum protein deficiency limits animal performance. The major outcome of increasing the postpartum protein supply was that more nutrients than the extra AA were partitioned to the mammary gland for

milk synthesis. Thus, even though the extra MP supply decreased with DIM for CAS, the treatment resulted in an increased milk production of 7.2 ± 1.3 kg/d and lactose yield of 436 ± 81 g/d, on average, during the first 4 wk of lactation, whereas the milk protein yield response to CAS decreased from 451 to 128 ± 95 g/d between 4 and 29 DIM.

Performance

Milk Production. The response observed in the current study was much greater than the 1 to 4 extra kilograms of milk observed with casein infusion in the

Table 7. Mammary uptake to output ratio of glucose equivalents and AA¹

Item	4 DIM		15 DIM		29 DIM		SEM ²	<i>P</i> -value		
	CTRL	CAS	CTRL	CAS	CTRL	CAS		Trt	DIM	Trt × DIM
Glucose	1.69	1.59	1.62	1.49	1.48	1.40	0.13	0.46	0.43	0.97
EAA	1.08	1.13	1.16	1.26**	1.09	1.21**	0.02	<0.01	<0.01	0.35
Group 1 EAA ³	1.02 ^u	1.00 ^u	1.00 ^u	1.05	0.98 ^u	1.06**	0.02	0.03	0.58	0.13
Group 2 EAA ⁴	1.10 ^u	1.17	1.22	1.29	1.17	1.23	0.04	0.10	0.04	0.99
His	1.18 ^u	0.98 ^u	1.02 ^u	1.20	1.01 ^u	1.31	0.10	0.22	0.48	0.13
Ile	1.19	1.23	1.31	1.34	1.29	1.29	0.05	0.65	0.22	0.94
Leu	1.06 ^u	1.11	1.12	1.18	1.08	1.12	0.04	0.17	0.27	0.97
Lys	1.06 ^u	1.24*	1.22	1.29	1.13	1.30**	0.04	0.01	0.14	0.39
Met	0.96 ^u	0.98 ^u	1.00 ^u	0.96 ^u	0.98 ^u	0.96 ^u	0.03	0.72	0.93	0.74
Thr	1.11	1.10	1.14	1.05 ^u	1.02 ^u	1.05 ^u	0.03	0.37	0.14	0.33
Trp	1.04 ^u	1.04 ^u	0.99 ^u	0.97 ^u	1.07 ^u	1.05 ^u	0.06	0.71	0.59	0.99
Val	1.12 ^u	1.12	1.29	1.38	1.25	1.27	0.05	0.39	<0.01	0.61
NEAA	0.77	0.78	0.69	0.73	0.63	0.75	0.03	0.14	0.15	0.33
Ala	2.07	1.41**	1.61	1.42	1.62	1.62	0.12	0.04	0.51	0.09
Asn	0.79	0.83	0.91	0.76	0.85	0.83	0.04	0.17	0.95	0.14
Asp	0.16	0.11	0.15	0.13	0.14	0.15	0.02	0.47	0.68	0.29
Cys	−0.08	1.10** ^u	1.35 ^u	0.98 ^u	1.11 ^u	0.88 ^u	0.26	0.80	0.20	0.03
Gln	1.36 ^u	1.67	1.14 ^u	1.32 ^u	0.91 ^u	1.21 ^u	0.22	0.33	0.17	0.91
Glu	0.56	0.44	0.53	0.45	0.47	0.48	0.06	0.46	0.89	0.30
Gly	0.21	0.38	0.26	0.40	0.44	0.50	0.19	0.62	0.53	0.94
Pro	0.40	0.51**	0.28	0.40**	0.28	0.33	0.02	0.01	<0.01	0.10
Ser	0.53	0.65	0.51	0.56	0.59	0.64	0.07	0.40	0.19	0.86
Total AA	0.91	0.94	0.90	0.96† ^u	0.83	0.95**	0.02	0.01	0.26	0.27

¹Treatments (Trt) were continuous abomasal infusion of water (CTRL) or Na-caseinate (CAS) from 1 to 29 DIM; casein protein infusion averaged 696 ± 1, 490 ± 9, and 212 ± 10 g/d at 4, 15, and 29 DIM, respectively.

²n = 4.

³Comprising the EAA His, Met, Phe (+Tyr), and Trp.

⁴Comprising the EAA Ile, Leu, Lys, and Val.

***P* ≤ 0.01, **P* ≤ 0.05, †*P* ≤ 0.10: symbols signify difference between LSM within DIM and rows.

^u*P* ≥ 0.10: symbol signifies uptake-to-output ratios not different from 1.00.

galactopoietic period (Cant et al., 1993; Mackle et al., 2000; Raggio et al., 2006). It was also interesting to note that the greater milk yield obtained with CAS persisted through the entire 4-wk period investigated (Figure 1c), even though the casein protein infusion had been reduced to 272 ± 17 g/d on average during the fourth week of lactation and total MP supply did not differ. Sørensen et al. (2006) observed increased apoptosis of mammary alveolar cells in the early postpartum period, and those authors hypothesized that this could reflect removal of nonfunctional and excess cell capacity. Based on the current work, it could be speculated that the observed persistency in milk synthesis may be caused by reduced postpartum apoptosis due to the greater postpartum protein supply providing more nutrients for mammary cell function. This may indicate that a higher set point for milk synthesis in the mammary gland had been achieved: this possible long-term effect of increasing the postpartum protein supply is intriguing and certainly warrants further investigation.

Milk production responses similar to the present have also been difficult to achieve when increasing the postpartum protein supply with feedstuffs. The obtained

early postpartum responses have ranged from −2 kg/d (Komaragiri and Erdman, 1997), no effect (Palmquist and Weiss, 1994; Socha et al., 2005), to 2 to 6 kg/d (Garcia-Bojalil et al., 1998; Xu et al., 1998; McCormick et al., 1999). The variation in effects observed on milk production may be related to several factors: (1) protein additions were relatively limited compared with the current study; (2) variations in ruminal degradability of used protein sources; (3) the rates of increment in extra protein supply were relatively slow due to use of TMR and therefore limited by DMI, as compared with the abomasal infusion used in the current study; (4) low palatability of protein sources used; and (5) the forage type used.

In comparison with the current response to increasing the postpartum protein supply, small production responses have been obtained when varying the dietary energy content of the postpartum diets (Rabelo et al., 2003; Guo et al., 2007; Larsen and Kristensen, 2010), and even profound negative effects have even been observed with abomasal glucose infusion (Larsen and Kristensen, 2009b). This indicates the existence of a window of opportunity in the very first days after par-

turation for inducing both positive and negative effects on the metabolic adaptation to lactation.

Protein and Energy Balances. The marginal utilization of the additional MP supply for milk protein was 60% at 4 DIM in the current study, considerably greater than the average of 21% (range 0 to 40%) observed with casein infusion during galactopoiesis when basal protein supply were below, at, or above requirement (Hanigan et al., 1998). In spite of the high marginal utilization of the additional MP, the postpartum whole body protein deficiency seemed to be alleviated, as the total MP utilization was greater than 100% with CAS at 4 DIM (<100% indicates mobilization). More EAA were available for all body functions when increasing the postpartum protein supply through increased arterial concentrations. This may benefit the immune system (Li et al., 2007), liver synthesis of export proteins (Connell et al., 1997), and the postpartum proliferation of visceral and liver tissue (Gibb et al., 1992).

The resulting increased need for energetic nutrients to support the greater milk synthesis with CAS was not supported by increased DMI; moreover, the excess total MP supply (not used for milk protein synthesis) theoretically covered a maximum of 30, 19, and 0% of the glucogenic precursors needed to cover the increased lactose yield at 4, 15, and 29 DIM, respectively. Even so, the greater circulating concentrations of urea indicate that more AA was catabolized for energetic purposes at the same time as more AA was used for milk protein synthesis. Therefore, the cows had to use the dietary nutrient supply more efficiently or mobilize more body depots or both. Increased arterial NEFA concentrations, numerically greater energy deficiency, and greater mammary BHBA uptake with CAS indicate that increased protein supply drove the cows to mobilize more body fat to support the increased energy need for milk production, a situation probably most pronounced during the first week of lactation. Greater mobilization of body fat may become a negative consequence of increasing the postpartum protein supply. Yet a substantial part of the mobilized FA was secreted in milk, as the milk fat yield was 470 ± 163 g/d greater with CAS at 4 DIM compared with CTRL without greater mammary acetate uptake; thus, the greater milk synthesis also provided a route for excretion of mobilized FA via milk fat.

Overall, the current experiment is a proof of the concept that postpartum protein deficiency imposes profound consequences to dairy cows and indicates that it has a greater effect than energy deficiency on performance. Yet, a need for future investigations elucidating how to transfer the effect obtained with abomasal infusion of high quality protein into practical feeding strategies exists.

Mammary Metabolism

Prepartum. Mammary uptake of nutrients in the prepartum period is generally not well described, and we are not aware of studies measuring blood flows and arterio-venous concentrations differences simultaneously in the prepartum period. Madsen et al. (2008) observed mammary release of BHBA and acetate, whereas mammary uptake of BHBA and acetate was observed in the current study. The difference between the studies may be related to the use of jugular blood as a substitute for arterial blood by Madsen et al. (2008). Investigating salivation using the water extraction method over the head (Storm et al., 2013), substantial uptake of BHBA and lactate, and no uptake of glucose across the head of dairy cows have been observed during rumination (A.C. Storm, Aarhus University, Denmark, personal communication). These observations show that care should be taken when using jugular blood as a substitute for arterial blood. The mammary gland was extracting all AA, except for Gly and Ser, from arterial plasma 14 d prepartum. To which extent the extracted AA was used for milk synthesis or cell proliferation is unknown; however, even though lactogenesis stage I had initiated, colostrum synthesis would be limited at 2 wk prepartum.

Mammary blood flow in dry cows is likely about half of that during lactation, as interpreted from observations of 2-fold differences at initiation of lactation in dairy cows (Braun et al., 2013) and goats (Davis et al., 1979), as well as at cessation of lactation in goats (Fleet and Peaker, 1978). Prepartum mammary blood and plasma flow in the current study would average 700 and 500 L/h, respectively, if assuming a 2-fold increment of blood flow in all veins draining the mammary gland from prepartum to 4 DIM. Using these flows, the mammary uptake of metabolites at 2 wk prepartum would average 1,330 mmol/h of O₂, 513 mmol/h of acetate, 93 mmol/h of glucose, 28 mmol/h of EAA, and 17 mmol/h of nonessential AA. Comparing estimates of prepartum mammary uptake of metabolites other than AA with the measured nutrient uptake at 4 DIM (Table 6) gives increments similar to those obtained in periparturient goats (Davis et al., 1979).

Postpartum AA Uptake. Mammary AA metabolism has typically been studied in the galactopoietic period by gradually changing from undersupply to oversupply of many or specific individual AA. During galactopoiesis, the mammary gland adapts to undersupply by (1) increasing supply by elevating blood flow, (2) increased extraction efficiency of AA from arterial supply, and (3) enhancing intramammary gland utilization toward protein synthesis for the AA taken up in excess of milk protein output (Mephram, 1982; Bequette et al.,

2000). In the current study, mammary metabolism was studied during establishment of lactation (lactogenesis stage I and II through to galactopoiesis), a period characterized by deficiency of protein and energetic nutrients induced by copious milk secretion in lactogenesis stage II.

Though not measured prepartum, the plasma flow would likely have increased greatly at 4 DIM relative to prepartum, as discussed previously. Plasma flow did not differ between treatments in absolute terms (L/h); hence, a similar number of AA transporters would likely be exposed to nutrients in blood. Yet, more nutrients were absorbed with CAS, thus indicating that neither plasma flow nor capacity of the transporter system, but supply of nutrients was limiting mammary nutrient absorption. Indeed, when expressed relative to milk yield, mammary plasma flow was numerically greater with CTRL in response to the postpartum protein deficiency, but this may simply be a mathematical consequence of the lower milk yield. Further in support of this was that the arterial concentration of metabolites normally considered to participate in regulating mammary blood flow were relatively unaffected by treatment at 4 DIM [e.g., His (Vanhatalo et al., 1999; Bequette et al., 2000; Huhtanen et al., 2002) glucose, BHBA, O₂, and acetate (Cant et al., 2003)].

Comparing arterial concentrations prepartum with 4 DIM for CTRL may help to identify the AA that become most deficient with the current diet when lactation was initiated: Leu, Lys, and Tyr decreased the most (21, 23, and 30% respectively). Initiation of lactation increased the mammary A-V concentration differences for all EAA as observed in goats (Mephram, 1982). The increased postpartum protein supply with CAS induced an even greater increment in mammary A-V concentration differences for Leu, Lys, and Tyr. The mammary A-V concentration differences of Leu, Lys, and Tyr at 4 DIM with CAS were similar to that observed at 29 DIM in the current experiment as well as in later lactation (Guinard and Rulquin, 1994; Lapierre et al., 2009). Altogether, these data would suggest that Lys and Leu would be the 2 limiting EAA in the postpartum period with current feedstuffs.

The greater net mammary uptake of EAA with CAS was roughly in line with the observed increment of milk protein yield; however, nuances in mammary AA metabolism could be observed when subdividing the EAA into group 1 (His, Met, Phe, Tyr, and Trp) or group 2 (Ile, Leu, Lys, and Val) based on mammary U:O ratios close to unity or U:O ratios greater than unity, respectively (Mephram, 1982; Lapierre et al., 2012). The U:O ratio for total group 1 EAA was greater than unity with CAS, but the effect was driven by His, as the other group 1 EAA did not differ from unity. The effect of

CAS on mammary U:O ratio for His is discussed later; thus, intramammary metabolism of the group 1 EAA were not affected by the overall postpartum protein deficiency. In contrast, the overall postpartum protein deficiency decreased the intramammary catabolism of the branched-chain AA and Lys, increasing the proportion of their uptake directed toward milk protein secretion. The mammary U:O ratio for Leu was generally in the low end of the studies reviewed by Lapierre et al. (2012), indicating low Leu supply in the current diet, typical of North European diets based on small grain cereals often combined with rapeseed meal. This also agrees with Finnish observations (Vanhatalo et al., 1999; Huhtanen et al., 2002).

The intramammary utilization of Lys was altered by the postpartum Lys deficiency, as indicated by the mammary U:O ratio for Lys at 4 DIM with CTRL not differing from unity, whereas it is usually greater than unity during galactopoiesis (Mephram, 1982; Lapierre et al., 2012). Indeed, Lys-deficient diets have been observed to decrease the mammary U:O ratio of Lys (Guinard and Rulquin, 1994; Lapierre et al., 2009), but not to levels as close to unity in the current study. This indicates that at very low supply of Lys combined with a strong driving metabolic demand for milk production, mammary uptake of Lys, on a net basis, is almost totally directed toward milk protein secretion. The reason for mammary U:O ratio not differing from unity for Lys at 4 DIM with CTRL may be related to the process of adaptation to lactation. In the prepartum cow, a net hepatic uptake of Lys has been observed (Larsen and Kristensen, 2013), likely related to catabolism of excess Lys. Likewise, net hepatic uptake of Lys was also observed at 4 DIM (Larsen and Kristensen, 2013), but the net hepatic uptake could not be related to excess Lys supply at this time. Hence, it could be speculated that, with respect to Lys, the process of adaptation to lactation involves both maturation of mammary metabolic pathways, allowing the metabolic flexibility in nonessential EAA synthesis from Lys, demonstrated by Lapierre et al. (2009), and accompanied by down-regulation of hepatic Lys uptake.

The postpartum general protein deficiency did not appear to induce His to limit postpartum milk synthesis, as arterial concentrations of His did not decrease between prepartum and 4 DIM in CTRL cows and the mammary A-V concentration difference of His at 4 DIM was not decreased by abomasal casein infusion. The reason for His not appearing as a limiting AA in the postpartum period may be linked to mobilization of carnosine and anserine-bound His in skeletal muscle. Usually, mammary His uptake equals output in milk protein (Lapierre et al., 2012), as observed with CTRL when lactation progresses. The intramammary utiliza-

tion of His seemed to be altered for His with CAS, as the U:O ratio increased from unity at 4 DIM to be greater than unity with the progressing lactation. With casein infusion during galactopoiesis, Raggio et al. (2006) did not observe mammary U:O ratio for His to increase above unity. The reason for the discrepancy is unknown.

Postpartum Uptake of Energetic Metabolites.

It was interesting to note that the mammary gland was releasing lactate on a net basis in the very first days postpartum with CTRL, but not with CAS at 4 DIM or at 15 and 29 DIM with both treatments. This could indicate that the mammary tissue express Cori cycling of glucogenic carbon only prepartum and in the very early postpartum period, as do other peripheral tissues (Kuhla et al., 2011; Larsen and Kristensen, 2013).

Even though the U:O ratio of glucose equivalents was unaffected by casein infusion, the U:O ratio of glucose equivalents was closer to unity (1.10 ± 0.08) when calculated on an incremental basis, indicating that the additional uptake of glucose with CAS was used for lactose synthesis with a relatively high efficiency. The intramammary greater partitioning of glucose to lactose synthesis would imply a greater uptake of other energetic nutrients; which was the case for lactate and BHBA but not for acetate. The present study does not allow calculation of mammary carbon balance, as long-chain FA, triglycerides, and glycerol were not analyzed in plasma or milk. Interestingly, Lemosquet et al. (2009) observed a numerical trend for negative mammary carbon balance when infusing casein as compared with propionate. This indicates that with high AA supply, the mammary gland has a greater dependency of energetic nutrients not typically measured or that the intramammary conversion efficiency of milk synthesis is increased.

CONCLUSIONS

The current results demonstrate that the postpartum protein deficiency can have substantial negative effect on milk production in dairy cows and indicates that MP supply is of greater importance than energy supply in this respect. In spite of a high utilization of the additional supplied MP for milk synthesis, more EAA were available for synthesis of plasma proteins, immune system, and proliferation of splanchnic tissues. Increasing the MP supply postpartum also seemed to increase the capacity of the mammary gland for milk synthesis as milk and lactose yields were still higher by 29 DIM despite similar MP supply at this time. Yet, future investigations are needed to delineate how to transfer the effect obtained with abomasal infusion into practical feeding strategies.

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