SUPPLEMENTAL MATERIAL

Table 1. Observed treatment amino concentrations of ^{15}N enriched medium

uM^2	Treatment AA Concentrations[1]					
	Trt1	Trt2	Trt3	Trt4	SEM	P-value
Ala	60.9	326.1	546.0	776.1	9.57	<0.001
Arg	9.9	65.9	126.4	185.6	1.95	<0.001
Asp	0.6	91.0	176.3	273.8	4.86	<0.001
Glu	15.7	157.5	294.8	450.9	8.01	<0.001
Gly	159.9	375.5	502.7	634.4	3.34	<0.001
Ile	21.6	124.7	217.3	317.6	4.91	<0.001
Leu	39.6	186.3	323.3	473.7	8.48	<0.001
Met	1.9	23.3	42.2	63.7	0.95	<0.001
Phe	8.4	53.9	97.1	144.3	2.18	<0.001
Pro	33.0	124.9	210.2	297.1	2.91	<0.001
Ser	26.4	193.6	318.5	513.0	25.78	<0.001
Thr	52.7	195.2	315.1	442.8	5.74	<0.001
Tyr	4.7	57.1	101.4	144.9	1.74	<0.001
Val	54.4	286.4	507.4	757.8	13.52	<0.001

[1]Treatment AA profiles mimicked typical lactating dairy cows and total AA concentrations were varied being 16% of in-vivo in Trt1 (0.36 mM), 100% of in-vivo Trt2 (2.30 mM), 186% of in-vivo Trt3 (4.28 mM), and 271% of in-vivo Tr4 (6.24 mM).
[2]AA concentrations are the summation of measured ^{12}C, ^{13}C, and ^{15}N masses of each AA.

Table 2. Observed treatment amino concentrations of ^{13}C enriched medium

	Treatment AA Concentrations[1]					
uM[2]	Trt1	Trt2	Trt3	Trt4	SEM	*P*-value
Ala	60.7^d	309.0^c	502.5^b	674.7^a	12.52	<0.001
Arg	12.6^d	80.8^c	135.0^b	192.6^a	5.55	<0.001
Asp	2.5^d	21.1^c	39.0^b	44.6^a	3.20	<0.001
Glu	6.1^d	96.0^c	139.1^b	181.4^a	5.23	<0.001
Gly	195.9^d	322.3^c	418.8^b	512.7^a	3.68	<0.001
Ile	23.9^d	129.2^c	208.7^b	286.5^a	5.62	<0.001
Leu	45.0^d	201.1^c	325.3^b	445.8^a	8.70	<0.001
Met	1.3^d	21.4^c	36.2^b	52.1^a	1.23	<0.001
Phe	44.6^d	119.7^c	179.7^b	241.0^a	4.09	<0.001
Pro	31.6^d	123.1^c	190.2^b	255.5^a	3.96	<0.001
Ser	34.1^d	141.9^c	228.6^b	276.9^a	12.80	<0.001
Thr	46.8^d	168.5^c	251.5^b	327.8^a	5.22	<0.001
Tyr	4.3^d	37.7^c	64.9^b	91.5^a	1.75	<0.001
Val	56.5^d	275.2^c	452.8^b	621.0^a	11.35	<0.001

[a-d]Least square means within a row with different superscripts differ ($P < 0.01$).

[1]Treatment AA profiles mimicked typical lactating dairy cows and total AA concentrations were varied being 16% of in-vivo in Trt1 (0.36 mM), 100% of in-vivo Trt2 (2.30 mM), 186% of in-vivo Trt3 (4.28 mM), and 271% of in-vivo (6.24 mM).

[2]AA concentrations are the summation of measured ^{12}C, ^{13}C, and ^{15}N masses of each AA.

Table 3. Effects of treatment on volume, cell, and protein associated measurements

Item	Treatment[1]						*P*-value			
	Trt1	Trt2	Trt3	Trt4	SEM	Treatment	Time	Linear[2]	Quadratic[2]
Starting plate weight, g	19.4	19.4	19.4	19.4	0.06	0.97	0.61	0.74	0.90
Plate weight before lysis, g	20.0	20.2	20.2	20.3	0.14	0.49	0.66	0.17	0.87
Cell weight from starting mass, g	0.64	0.78	0.82	0.93	0.14	0.51	0.73	0.14	0.90
Cell weight from ending mass, g	0.55	0.65	0.77	0.82	0.17	0.66	0.60	0.20	0.89
Harvested cell weight, g	0.29	0.32	0.34	0.39	0.03	0.09	0.40	0.01	0.83
Protein weight, g	0.074	0.086	0.093	0.094	0.002	<0.001	0.19	<0.001	0.02
Cells (1000) / mL	1371	1200	1508	1675	126	0.09	-	0.03	0.17
Cells (1000) / total	2148	1871	2438	2710	183	0.04	-	0.01	0.13
Cell, ng	0.73	0.84	0.67	0.60	0.06	0.07	-	0.03	0.13
Protein, ug/mL	3602	4056	4232	3498	738	0.77	-	0.96	0.37

[1]Treatment AA profiles mimicked typical lactating dairy cows and total AA concentrations were varied being 16% of in-vivo in Trt1 (0.36 m*M*), 100% of in-vivo Trt2 (2.30 m*M*), 186% of in-vivo Trt3 (4.28 m*M*), and 271% of in-vivo (6.24 m*M*).
[2]Linear and quadratic contrasts of treatment AA concentrations effects.

Table 4. Effect of treatment on intracellular free amino acid mass

umol[2]	Treatment[1]				SEM	P-value			
	Trt1	Trt2	Trt3	Trt4		Trt	Time	Linear[3]	Quadratic[3]
Ala	183	846	1054	1180	18	<0.001	<0.001	<0.001	<0.001
Arg	1	11	28	34	5	<0.001	0.44	<0.001	0.71
Asp	54	179	155	185	9	<0.001	<0.001	<0.001	<0.001
Glu	1009	1426	1443	1581	37	<0.001	0.18	<0.001	<0.001
Gly	835	892	899	964	16	<0.001	0.08	<0.001	0.78
Ile	17	107	142	157	3	<0.001	<0.001	<0.001	<0.001
Leu	36	159	208	230	4	<0.001	<0.001	<0.001	<0.001
Met	3	20	44	59	2	<0.001	<0.001	<0.001	0.58
Phe	53	100	101	103	2	<0.001	<0.001	<0.001	<0.001
Pro	142	675	727	780	14	<0.001	<0.001	<0.001	<0.001
Ser	126	248	283	394	11	<0.001	<0.001	<0.001	0.60
Thr	106	307	381	452	7	<0.001	<0.001	<0.001	<0.001
Tyr	35	86	95	104	2	<0.001	<0.001	<0.001	<0.001
Val	46	270	326	332	6	<0.001	<0.001	<0.001	<0.001

[1]Treatment AA profiles mimicked typical lactating dairy cows and total AA concentrations were varied being 16% of in-vivo in Trt1 (0.36 mM), 100% of in-vivo Trt2 (2.30 mM), 186% of in-vivo Trt3 (4.28 mM), and 271% of in-vivo (6.24 mM).
[2]Mass includes ^{12}C, ^{13}C, and ^{15}N mass of each AA.
[3]Linear and quadratic contrasts of treatment AA concentrations effects.

Table 5. Effect of treatment on intracellular protein bound amino acid mass

| millimole[2] | Treatment[1] | | | | SEM | P-value |
	Trt1	Trt2	Trt3	Trt4		Trt
Ala	2.6	2.1	2.1	2.0	0.04	<0.001
Arg	1.4	1.6	1.5	1.7	0.13	0.20
Asp	6.0	5.8	5.8	5.8	0.10	0.11
Glu	10.5	11.4	12.0	11.7	0.21	<0.001
Gly	0.4	0.4	0.4	0.4	0.01	0.01
Ile	2.6	3.0	3.2	3.2	0.08	<0.001
Leu	5.5	6.4	7.0	6.7	0.23	<0.001
Met	1.1	1.2	1.3	1.3	0.05	0.01
Phe	2.5	2.9	3.1	3.0	0.06	<0.001
Pro	2.1	2.3	2.4	2.4	0.06	<0.001
Ser	0.7	0.6	0.6	0.6	0.02	<0.001
Thr	1.4	1.2	1.1	1.1	0.02	<0.001
Tyr	0.7	0.5	0.5	0.5	0.01	<0.001
Val	3.0	3.5	3.7	3.7	0.09	<0.001

[1]Treatment AA profiles mimicked typical lactating dairy cows and total AA concentrations were varied being 16% of in-vivo in Trt1 (0.36 mM), 100% of in-vivo Trt2 (2.30 mM), 186% of in-vivo Trt3 (4.28 mM), and 271% of in-vivo (6.24 mM).
[2]Mass includes ^{12}C, ^{13}C, and ^{15}N mass of each AA.

Table 6. Nonessential amino acid parameter estimates (min^{-1}) derived by fitting the model to the observed treatment data[1]

	Treatment, min^{-1}											
	Trt1			Trt2			Trt3			Trt4		
Item[2]	Estimate	95% CI	CV	Estimate	95% CI	CV	Estimate	95% CI	CV	Estimate	95% CI	CV
Ala												
k_{nAAnKA}	0.012[b]	0.008-0.016	18.4	0.044[a]	0.040-0.049	5.1	0.046[a]	0.043-0.049	3.37	0.043[a]	0.039-0.046	4.6
k_{nAAOx}	0.070[a]	0.056-0.083	9.8	0.029[b]	0.019-0.040	18.2	0.021[bc]	0.014-0.027	15.3	0.012[c]	0.007-0.016	19.6
$k_{nAAtfAA}$	0.003	0-0.006	62.5	0.001	0-0.002	83.7	0.001	0.000-0.001	50	0.001	0.000-0.001	28.7
k_{nAAxAA}	0.023[c]	0.018-0.027	10.6	0.053[b]	0.045-0.061	7.7	0.067[ab]	0.061-0.074	4.88	0.070[a]	0.063-0.078	5.7
$k_{synthesis}$	7.86	6.33-9.40	9.9	8.90	4.75-13.06	23.8	9.01	5.98-12.04	17.2	7.21	5.01-9.42	15.6
k_{xAAnAA}	0.026[ab]	0.023-0.029	6.7	0.032[a]	0.029-0.035	4.4	0.025[a]	0.024-0.027	3.17	0.021[b]	0.019-0.023	4.8
Asp												
k_{xAAOx}	0.015	0.000-0.029	50.0	0.005	0-0.015	115.1	0.009	0-0.021	66.4	0.010	0.001-0.018	43.3
$k_{nAAtfAA}$	0.007	0.005-0.008	11.7	0.005	0.004-0.006	8.0	0.006	0.003-0.009	25.9	0.005	0.004-0.006	12.9
k_{nAAxAA}	0.001	0.001-0.001	7.1	0.002	0.001-0.003	30.7	0.002	0.002-0.003	17.8	0.002	0.002-0.003	11.1
$k_{synthesis}$	0.80[b]	0.15-1.46	41.4	1.70[ab]	0.94-2.47	23.0	2.39[a]	1.49-3.30	19.4	2.55[a]	2.06-3.03	9.8
k_{xAAnAA}	0.005[a]	0.003-0.008	20.7	0.002[ab]	0.001-0.004	27.3	0.002[ab]	0.001-0.003	20.8	0.002[b]	0.001-0.002	15.2
Glu												
k_{xAAOx}				0.015[b]	0.013-0.018	8.0	0.019[ab]	0.017-0.021	5.01	0.024[a]	0.022-0.027	5.1
$k_{nAAtfAA}$	0.003[c]	0.002-0.003	9.3	0.006[b]	0.006-0.007	4.2	0.019[a]	0.019-0.020	1.86	0.008[b]	0.007-0.009	3.8
k_{nAAxAA}	0.000[b]	0.000-0.001	15.2	0.003[a]	0.002-0.003	10.0	0.002[ab]	0.001-0.003	25.3	0.002[ab]	0.001-0.004	26.7
$k_{synthesis}$	3.23[c]	2.56-3.90	10.5	19.60[b]	17.33-21.88	5.9	23.54[ab]	21.69-25.39	4.01	24.86[a]	22.59-27.14	4.7
k_{xAAnAA}	0.010[a]	0.009-0.011	4.8	0.004[b]	0.003-0.006	14.7	0.002[b]	0.001-0.003	28.2	0.003[b]	0.002-0.004	13.4
Gly												
k_{xAAOx}	0.014[b]	0.009-0.018	16.6	0.027[a]	0.023-0.032	8.9	0.017[b]	0.014-0.020	10.1	0.009[b]	0.005-0.012	22.3
$k_{nAAtfAA}$	0.000	0.000-0.000	40.6	0.0002	0.000-0.000	17.8	0.000	0.000-0.000	31.7	0.000	0.000-0.000	22.2
k_{nAAxAA}	0.031[ab]	0.026-0.036	8.1	0.032[a]	0.029-0.036	5.6	0.024[b]	0.022-0.026	4.05	0.020[c]	0.019-0.021	3.0
$k_{synthesis}$	12.20[ab]	9.76-14.64	10.2	13.34[a]	11.32-15.35	7.7	9.30[b]	7.89-10.72	7.75	6.11[c]	4.57-7.66	12.9
k_{xAAnAA}	0.016[a]	0.013-0.019	9.8	0.011[a]	0.010-0.013	5.6	0.007[b]	0.006-0.008	4.52	0.004[c]	0.004-0.005	5.1
Pro												
k_{nAAnKA}	0.003	0.001-0.006	38.3	0.004	0.002-0.006	27.0						
k_{xAAOx}	0.005[ab]	0.003-0.007	19.0	0.007[a]	0.006-0.009	11.1	0.004[a]	0.003-0.006	13.4	0.002[b]	0.001-0.003	25.3

	Trt1			Trt2			Trt3			Trt4		
$k_{nAAtfAA}$	0.005[a]	0.004-0.005	6.1	0.002[b]	0.002-0.002	4.6	0.002[b]	0.002-0.002	3.59	0.002[b]	0.002-0.002	3.0
k_{nAAxAA}	0.010[c]	0.009-0.012	9.2	0.021[a]	0.019-0.023	4.8	0.020[a]	0.018-0.021	3.49	0.015[b]	0.014-0.016	3.3
k_{xAAnAA}	0.011[b]	0.010-0.012	5.6	0.018[a]	0.017-0.019	3.4	0.010[b]	0.010-0.011	2.57	0.006[c]	0.006-0.007	2.3
Ser												
k_{xAAOx}	0.045[ab]	0-0.102	64.0	0.042[a]	0.033-0.050	10.1	0.032[ab]	0.021-0.044	18.6	0.021[a]	0.014-0.028	17.4
$k_{nAAtfAA}$	0.003	0-0.010	122.5	0.001	0.000-0.001	44.7				0.000	0.000-0.001	59.6
$k_{nAAtsAA}$				0.000	0.000-0.001	46.1						
k_{nAAxAA}	0.037[b]	0.015-0.058	29.5	0.069[ab]	0.050-0.088	13.9	0.067[ab]	0.048-0.087	14.7	0.075[a]	0.064-0.086	7.7
$k_{synthesis}$	5.73	0.63-10.84	45.5	7.01	5.85-8.17	8.5	6.66	4.97-8.38	12.9	7.28	5.88-8.69	9.8
k_{xAAnAA}	0.026	0.012-0.041	27.6	0.027	0.020-0.034	12.4	0.017	0.013-0.022	13.8	0.017	0.014-0.020	7.6
Tyr												
k_{xAAOx}				0.015[a]	0.012-0.019	11.2	0.011[a]	0.009-0.014	12	0.005[b]	0.002-0.008	27.0
$k_{nAAtfAA}$				0.001	0.000-0.003	42.2				0.001	0.000-0.003	65.8
k_{nAAxAA}	0.024[b]	0.016-0.032	17.3	0.042[b]	0.030-0.053	14.1	0.063[a]	0.055-0.072	6.99	0.064[a]	0.055-0.074	7.7
$k_{synthesis}$	0.09[b]	0.045-0.132	25.2	0.26[a]	0.176-0.339	16.1	0.20[a]	0.148-0.259	13.8			
k_{xAAnAA}	0.013	0.008-0.018	20.5	0.013	0.010-0.016	13.2	0.012	0.011-0.014	6.64	0.009	0.008-0.011	8.6

[a-c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatment AA profiles mimicked typical lactating dairy cows and total AA concentrations were varied being 16% of in-vivo in Trt1 (0.36 mM), 100% of in-vivo Trt2 (2.30 mM), 186% of in-vivo Trt3 (4.28 mM), and 271% of in-vivo (6.24 mM).

[2]Rate constants: k_{nAAOx} = oxidation, $k_{nAAtfAA}$ = fast protein turnover, $k_{nAAtsAA}$ = slow protein turnover, k_{nAAxAA} = efflux, k_{xAAnAA} = uptake, $k_{synthesis}$ = fractional synthesis, and k_{nAAnKA} = transamination.

[3]95% CI derived from Markov Chain Monte Carlo simulation (n=5000 runs).

Table 7. Fit statistics for predictions of observed essential amino acid isotope ratios by amino acid and treatment[1]

Item	Statistic[2]	E_{nAA}^{13C}	E_{xAA}^{13C}	E_{nAA}^{15N}	E_{xAA}^{15N}	E_{tAA}^{13C}	E_{tAA}^{15N}
				Isotope Ratios[3]			
Ala							
Trt1	CCC	0.62	0.81	0.62	0.47	0.17	0.00
	RMSE	15.8	16.7	11.9	9.3	3.1	4.6
Trt2	CCC	0.91	0.99	0.98	0.96	0.13	0.03
	RMSE	11.6	4.9	6.3	5.9	3.0	3.8
Trt3	CCC	0.93	0.99	1.00	0.96	0.31	0.05
	RMSE	10.8	4.0	3.2	5.9	2.1	3.8
Trt4	CCC	0.93	0.99	0.96	0.98	0.51	0.04
	RMSE	11.1	4.1	9.7	4.8	1.8	3.5
Asp							
Trt1	CCC	0.55	0.23	0.81	0.08	0.77	0.01
	RMSE	50.1	46.9	28.2	8.8	2.1	1.0
Trt2	CCC	0.25	0.83	0.71	0.49	0.94	0.05
	RMSE	39.2	34.0	37.0	19.5	1.6	1.5
Trt3	CCC	0.33	0.89	0.82	0.21	0.81	0.18
	RMSE	32.9	23.5	31.5	13.8	2.9	2.0
Trt4	CCC	-0.03	0.87	0.83	0.35	0.86	-0.04
	RMSE	34.0	28.4	25.1	17.9	2.7	2.2
Glu							
Trt1	CCC	0.88	0.48	0.77	0.05	0.97	-0.01
	RMSE	21.9	62.7	5.5	6.6	2.5	1.5
Trt2	CCC	0.44	0.93	0.88	0.67	0.96	0.20
	RMSE	52.7	25.3	14.1	12.5	3.6	1.4
Trt3	CCC	0.18	0.92	0.89	0.68	0.86	0.32
	RMSE	75.2	26.9	13.9	9.9	7.0	1.8
Trt4	CCC	0.41	0.87	0.88	0.63	0.91	0.00
	RMSE	65.4	31.8	15.2	10.2	6.5	1.9
Gly							
Trt1	CCC	0.83	0.94	0.67	0.94	0.27	-0.02
	RMSE	5.1	8.2	4.0	5.5	1.0	1.2
Trt2	CCC	0.90	0.96	0.90	0.94	0.66	0.06
	RMSE	8.4	3.9	4.3	7.8	0.9	1.4
Trt3	CCC	0.96	0.96	0.95	0.96	0.38	0.01
	RMSE	6.5	2.3	3.5	4.4	1.6	2.3
Trt4	CCC	0.97	0.98	0.97	0.98	0.58	-0.01
	RMSE	5.1	1.0	2.7	2.3	1.2	2.0
Pro							
Trt1	CCC	0.88	0.66	0.84	0.57	0.97	0.00
	RMSE	28.1	14.1	5.3	14.0	10.0	9.3

		E_{nAA}^{13C}	E_{xAA}^{13C}	E_{nAA}^{15N}	E_{xAA}^{15N}	E_{tAA}^{13C}	E_{tAA}^{15N}
Trt2	CCC	0.97	0.95	0.96	0.98	0.96	0.01
	RMSE	20.9	6.6	4.9	8.5	10.3	9.6
Trt3	CCC	0.99	0.97	0.98	0.98	0.99	0.02
	RMSE	11.5	3.3	3.6	7.1	6.2	5.8
Trt4	CCC	0.99	0.96	0.94	0.98	0.98	0.05
	RMSE	14.6	2.7	6.3	6.5	6.6	4.2
Ser							
Trt1	CCC	0.59	0.90	0.13	0.83	0.09	-0.01
	RMSE	15.2	10.9	5.6	5.2	3.3	1.0
Trt2	CCC	0.40	0.90	0.79	0.97	0.16	0.02
	RMSE	22.4	8.1	7.3	5.1	4.2	1.6
Trt3	CCC	0.52	0.72	0.83	0.89	NA	NA
	RMSE	24.0	8.5	8.5	8.7	3.9	2.2
Trt4	CCC	0.85	0.77	0.91	0.96	0.14	-0.06
	RMSE	13.3	9.7	6.3	5.2	3.1	1.9
Tyr							
Trt1	CCC	0.75	0.71	0.05	0.63		
	RMSE	10.62	21.08	6.22	3.05	8.20	8.29
Trt2	CCC	0.84	0.80	0.91	0.94	0.18	0.02
	RMSE	6.92	3.68	10.45	7.67	2.36	2.57
Trt3	CCC	0.87	0.84	0.99	0.96		
	RMSE	4.20	1.56	5.29	5.63	2.71	3.09
Trt4	CCC	0.77	0.49	0.97	0.98	0.15	0.02
	RMSE	4.65	1.76	10.02	4.03	2.41	2.79

[1]Treatment AA profiles mimicked typical lactating dairy cows and total AA concentrations were varied being 16% of in-vivo in Trt1 (0.36 mM), 100% of in-vivo Trt2 (2.30 mM), 186% of in-vivo Trt3 (4.28 mM), and 271% of in-vivo (6.24 mM).

[2]Test statistics: RMSPE=root mean square prediction error, CCC=concordance correlation coefficient.

[3]Isotope ratios: E_{nAA}^{13C}=^{13}C/^{12}C area ratio of free intracellular AA, E_{xAA}^{13C}=^{13}C/^{12}C area ratio of free media AA, E_{nAA}^{15N}=^{15}N/^{12}C area ratio of free intracellular AA, E_{xAA}^{15N}=^{15}N/^{12}C area ratio of free media AA, E_{tAA}^{13C}=^{13}C/^{12}C area ratio of protein bound AA, and E_{tAA}^{15N}=^{15}N/^{12}C area ratio of protein bound AA.

Table 8. Nonessential amino acid flux predictions derived from the parameterized model and the treatment data[1]

| | Amino Acid Fluxes, nmol/min[2] | | | | | | | | | | | | | | | | | | | | |
| | Net Uptake | | | Uptake | | | Exit | | | Turnover | | | Transamination | | | Oxidation | | | Synthesis | | |
Item	Mean	5%	95%	Mean	5%	95%	Mean	5%	95%	Mean	5%	95%	Mean	5%	95%	Mean	5%	95%	Mean	5%	95%
Ala																					
Trt1	2.4[c]	2.2	2.6	6.8[c]	6.0	7.6	4.4[d]	3.5	5.2	0.5	0.0	1.2	2.3[c]	1.4	3.2	13.4[b]	11.2	15.6	12.0	9.8	14.1
Trt2	6.4[a]	5.9	7.0	51.2[b]	47.1	55.3	44.7[c]	40.6	48.9	0.6	0.0	1.4	37.2[b]	33.4	41.1	24.5[a]	17.3	31.6	17.3	9.7	24.9
Trt3	4.0[b]	3.2	4.8	74.3[a]	70.0	78.6	70.3[b]	66.0	74.7	0.7	0.1	1.2	47.7[a]	44.1	51.2	21.6[a]	16.0	27.3	18.0	12.3	23.6
Trt4	-0.2[d]	-1.9	1.4	82.9[a]	75.4	90.5	83.2[a]	75.9	90.5	0.7	0.4	1.1	50.3[a]	45.8	54.9	13.9[ab]	9.2	18.7	14.8	10.6	19.0
Asp																					
Trt1	0.4	0.3	0.5	0.5[b]	0.4	0.6	0.0[b]	0.0	0.0	0.3[b]	0.2	0.4				0.7	0.1	1.3	0.8[b]	0.2	1.4
Trt2	0.5	0.2	0.7	0.8[ab]	0.5	1.1	0.3[a]	0.3	0.4	0.8[a]	0.7	1.0				0.8	-0.1	1.7	1.7[ab]	1.0	2.4
Trt3	0.6	0.3	1.0	1.0[a]	0.7	1.4	0.4[a]	0.3	0.5	1.1[a]	0.6	1.5				1.6	0.1	3.1	2.4[a]	1.5	3.3
Trt4	0.7	0.4	1.0	1.2[a]	0.9	1.5	0.5[a]	0.4	0.5	1.0[a]	0.7	1.2				2.0	0.9	3.0	2.5[a]	2.1	3.0
Glu																					
Trt1	0.8[a]	0.7	0.9	1.2	1.1	1.3	0.4[b]	0.3	0.5	2.7[d]	2.2	3.2							3.3[c]	2.6	4.0
Trt2	-1.7[b]	-2.2	-1.3	1.7	1.2	2.2	3.5[a]	2.9	4.0	8.7[c]	7.9	9.5				21.2[b]	18.6	23.9	20.1[b]	17.9	22.2
Trt3	-1.8[b]	-2.8	-0.9	0.9	0.3	1.6	2.7[a]	1.4	4.1	25.6[a]	24.6	26.5				25.7[b]	23.2	28.2	24.2[a]	22.4	26.1
Trt4	-1.2[b]	-2.5	0.1	2.0	1.3	2.7	3.2[a]	1.5	4.9	10.5[b]	9.6	11.3				32.2[a]	29.0	35.4	25.6[a]	23.3	28.0
Gly																					
Trt1	-8.4[c]	-9.3	-7.5	16.8[bc]	13.7	19.8	25.2[ab]	21.7	28.6	0.1	0.0	0.3				11.0[bc]	7.6	14.5	18.6[ab]	15.1	22.1
Trt2	-3.1[b]	-3.6	-2.5	24.4[ab]	21.7	27.1	27.5[a]	24.7	30.2	0.2	0.1	0.2				23.5[a]	19.6	27.3	24.2[a]	20.6	27.9
Trt3	-0.9[a]	-1.5	-0.3	21.4[b]	19.7	23.2	22.3[b]	20.8	23.9	0.1	0.1	0.2				15.6[b]	12.8	18.4	16.9[b]	14.5	19.3
Trt4	-2.7[b]	-3.7	-1.7	16.8[c]	15.3	18.3	19.5[c]	18.5	20.5	0.2	0.1	0.2				8.5[c]	5.1	11.9	10.9[c]	8.3	13.5
Pro																					
Trt1	0.4[a]	0.3	0.5	1.9[c]	1.7	2.1	1.5[c]	1.2	1.7	0.7[c]	0.6	0.8	0.5[b]	0.1	0.8	0.7[c]	0.5	0.9			
Trt2	-0.1[b]	-0.4	0.3	12.8[a]	12.0	13.6	12.8[ab]	11.9	13.7	1.1[b]	1.0	1.2	2.3[a]	1.1	3.4	4.3[a]	3.6	5.1			
Trt3	-1.0[c]	-1.3	-0.6	11.9[a]	11.3	12.5	12.9[a]	12.2	13.5	1.2[b]	1.1	1.3				2.9[b]	2.3	3.5			
Trt4	-1.5[c]	-1.9	-1.0	9.8[b]	9.4	10.2	11.3[b]	10.7	11.9	1.5[a]	1.4	1.6				1.6[bc]	0.9	2.3			
Ser																					
Trt1	-0.4[b]	-0.6	-0.2	5.3[b]	2.6	8.0	5.7[c]	2.9	8.5	0.4	-0.5	1.4				7.0	-0.4	14.5	9.1	1.7	16.5
Trt2	0.9[a]	0.6	1.3	21.5[a]	16.9	26.2	20.6[b]	16.0	25.2	0.2	0.0	0.4				12.4	10.4	14.5	12.9	10.9	15.0

Trt3	0.5[ab]	-0.3	1.3	22.9[a]	16.9	28.9	22.4[ab]	16.4	28.4				10.8	7.5	14.1	12.4	9.4	15.4
Trt4	-3.1[c]	-4.3	-2.0	28.8[a]	24.7	33.0	31.9[a]	27.8	36.1	0.2	0.0	0.4	8.9	6.0	11.7	13.6	11.3	16.0
Tyr																		
Trt1	-0.3[b]	-0.4	-0.3	0.4[c]	0.2	0.6	0.8[c]	0.5	1.0							0.1[b]	0.1	0.2
Trt2	-0.1[a]	-0.2	0.0	3.0[b]	2.2	3.8	3.1[b]	2.3	3.9	0.1	0.0	0.2	1.2[a]	1.0	1.4	0.6[a]	0.4	0.8
Trt3	-0.2[ab]	-0.3	-0.1	4.9[a]	4.3	5.5	5.2[a]	4.6	5.8				0.9[a]	0.8	1.1	0.5[a]	0.4	0.7
Trt4	-0.4[ab]	-0.6	-0.2	4.9[a]	4.1	5.8	5.3[a]	4.6	6.1	0.1	0.0	0.2	0.4[b]	0.2	0.6			

[a-d]Means within a column with different superscripts differ ($P < 0.05$).

[1]Treatment AA profiles mimicked typical lactating dairy cows and total AA concentrations were varied being 16% of in-vivo in Trt1 (0.36 mM), 100% of in-vivo Trt2 (2.30 mM), 186% of in-vivo Trt3 (4.28 mM), and 271% of in-vivo (6.24 mM).

[2]Average flux over 60 minutes using derived rate constants and experimental data.

[3]5% and 95% confidence interval standard error represents the standard deviation of 1000 simulated model runs conducted using randomly drawn parameters from Markov Chain Monte Carlo derived parameter posteriors.

Table 9. Media, intracellular free, and intracellular protein bound nonessential amino acid pool turnover per hour by treatment and amino acid[1]

	Treatment Pool Turnover, % / hour			
	Trt1	Trt2	Trt3	Trt4
Ala				
Extracellular Free AA	100.7	166.7	143.8	123.9
Intracellular Free AA	600.6	495.2	534.1	499.9
Fast Protein Bound AA	40.7	47.3	52.3	57.6
Asp				
Extracellular Free AA	2.7	5.9	4.3	4.0
Intracellular Free AA	218.4	135.9	179.8	152.7
Fast Protein Bound AA	6.2	12.1	16.3	16.7
Glu				
Extracellular Free AA	18.0	53.3	31.5	27.7
Intracellular Free AA	42.9	138.4	238.5	181.4
Fast Protein Bound AA	54.3	137.9	422.7	162.9
Gly				
Extracellular Free AA	146.6	77.2	44.5	30.6
Intracellular Free AA	263.4	342.9	252.5	169.0
Fast Protein Bound AA	3.6	3.8	3.4	4.1
Pro				
Extracellular Free AA	50.7	106.6	65.6	43.2
Intracellular Free AA	106.1	138.9	119.7	91.4
Fast Protein Bound AA	18.2	20.7	21.9	26.2
Ser				
Extracellular Free AA	170.7	154.8	101.2	113.1
Intracellular Free AA	570.1	696.7	636.2	599.4
Fast Protein Bound AA	129.3	2.8		16.2
Tyr				
Extracellular Free AA	130.9	80.4	76.4	59.5
Intracellular Free AA	111.4	297.7	401.9	367.1
Fast Protein Bound AA		36.4		29.2

[1]Treatment AA profiles mimicked typical lactating dairy cows and total AA concentrations were varied being 16% of in-vivo in Trt1 (0.36 mM), 100% of in-vivo Trt2 (2.30 mM), 186% of in-vivo Trt3 (4.28 mM), and 271% of in-vivo (6.24 mM).