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Effect of Intraruminal Propionic Acid Infusion on Metabolism of Mesenteric- and Portal-Drained Viscera in Growing Steers Fed a Forage Diet: II. Ammonia, Urea, Amino Acids, and Peptides^{1,2}

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ABSTRACT: This experiment investigated the effect of intraruminal infusion of propionic acid on the net flux of nitrogenous compounds across the mesenteric- (MDV) and portal-(PDV) drained viscera of seven Friesian steers, average BW 127 kg (SEM 4.6), fed a grass-pellet diet. Each received by random allocation 0 (control), .5, or 1.0 mol of propionic acid/d for 7 d. Blood flow in mesenteric and portal veins was determined by downstream dilution of *p*-aminohippuric acid in order to determine net appearance rates across the gastrointestinal tissues. Net urea and ammonia flux was unaffected by propionic acid supply. Circulating plasma free amino acid concentrations were increased ($P < .05$) by propionic acid infusion (2,235, 2,428, and 2,427, error mean square [EMS]

44,370 μM , for control, .5, and 1.0 mol of propionic acid/d, respectively). Net amino acid flux rates were increased at the highest rate of propionic acid infusion across MDV and PDV (4.66, 3.69, and 6.11, EMS 2.98 mol/d for MDV [$P < .05$] and 2.98, 2.45, and 3.73, EMS 1.69 mol/d for PDV [$P < .10$] for control, .5, and 1.0 mol of propionic acid/d respectively). Positive venous-arterio concentration differences for peptide-bound amino acids (PBAA) across the MDV and PDV indicated net appearance across the gastrointestinal tissues, but this was not affected by propionic acid infusion. The data show that amino acid flux across postruminal tissues can be influenced by ruminal propionic acid supply and that this does not affect PBAA appearance.

Key Words: Propionic Acid, Amino Acids, Peptides, Portal Vein, Net Flux

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Introduction

The role of the gastrointestinal tract and the associated microflora in the digestion and metabolism of nitrogenous feed components is well established. In particular, the effect of diet composition on ammonia and urea production and metabolism has received considerable attention (for review see Parker et al., 1995). Several studies have shown that ruminant animals fed high-N-containing forages have a reduced efficiency of protein deposition or milk output, and that this may be improved by either supply of extra

protein or fermentable carbohydrate. This inefficiency may be due to impaired supply of absorbed amino acids through ruminal production of ammonia (Beever et al., 1985). In addition, substantial metabolism of amino acids within the gastrointestinal tissues further reduces the availability of amino-N to peripheral tissues (Tagari and Bergman, 1978). Their study demonstrated that between 20 and 70% of the individual amino acids entering the small intestine did not arrive in the portal vein; these amino acids were required both to maintain the high rates of protein turnover observed in the gastrointestinal tract (Attaix et al., 1988; Lobley, 1991) and for use as energy-yielding substrates within the mucosa. In conjunction with measurements of VFA and glucose metabolism (Seal and Parker, 1994), this experiment was designed to investigate the effects of changing ruminal propionate supply on flux of nitrogenous compounds across the mesenteric- and portal-drained visceral tissues. Results from part of this experiment have been presented as an abstract (Seal and Parker, 1991a).

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Materials and Methods

Full details of the experimental design have been previously described in a companion paper (Seal and Parker, 1994). Briefly, seven Friesian steers with an average BW of 127 kg (SEM 4.6) at the start of the experiment were used. Each had been fitted with a small ruminal fistula (40 mm i.d.) and chronic indwelling silastic catheters in the portal vein, mesenteric vein, and a carotid artery as described previously (Seal et al., 1992). The steers were housed in individual stalls and were fed a grass-pellet diet by continuous feeders in 24 equal lots throughout the day (grams/kilogram DM crude protein, 194; crude fiber, 234; GE, 4.73 Mcal/kg of DM) at 28 g of DM/kg BW, calculated on the basis of ME intake from ARC tables (ARC, 1984) to give a 700 g/d weight gain. There were no feed refusals during the experiment. All steers received each treatment consisting of 7 d of continuous intraruminal infusions of 1.44 L of water (control), or the same volume containing either .5 or 1.0 mol of propionic acid/d. Eight steers were prepared in pairs and treatments were allocated in random order to each steer. One steer completed only one treatment period and has not been included in the analysis.

Sampling Procedure. Blood flow in the anterior mesenteric and portal veins was determined by downstream dye dilution using *p*-aminohippuric acid (**PAH**; Katz and Bergman, 1969) infused into the distal mesenteric vein for the determination of net nutrient flux rates across the gastrointestinal tract. Plasma flow was determined as the product of whole blood flow and (1 - hematocrit). Replicate observations for each steer on the three treatments were obtained during parallel isotope infusion as described previously (Seal and Parker, 1994).

Analytical Methods

Blood Metabolites and *p*-Aminohippuric Acid. Ammonia and urea concentrations were determined enzymically using a Roche Cobas Mira Clinical Analyser (Roche Diagnostics, Welwyn Garden City, U. K.). Plasma free amino acid (**FAA**) concentrations were determined in samples bulked for each sampling period by reversed-phase HPLC after filtration through 10K molecular weight filters (Millipore U.K. Ltd., Watford, U. K.) and pre-column derivatization with phenylisothiocyanate using a Waters Pico-Tag system (Waters Chromatography Division, Millipore, Milford, MA).

Low-Molecular-Weight Peptides. Low-molecular-weight peptides in plasma were isolated by reversed-phase HPLC using a slight modification of a previously published procedure (Seal and Parker, 1991b) in samples from three steers using one collection during control and 1.0 mol/d infusions only. Filtrates from plasma filtered through 10K molecular weight filters were separated using a Spherisorb C18 ODS 5

reversed-phase column (5 mm × 250 mm, Phase Separations Ltd., Queensferry, U. K.) on a Waters dual-pump HPLC system with autoinjection. Filtrate (200 μL) was applied to the column and the peptides separated with an aqueous/acetonitrile gradient, 100% buffer A (.1% trifluoroacetic acid [**TFA**] in double-distilled water) to 60% buffer B (.1% TFA in acetonitrile) over 45 min. The column was cleaned by flushing with 100% buffer B for 5 min and re-equilibrated in 100% buffer A for 25 min before the next sample. Eluate from the column passed through a detector at 225 nm and was collected manually into four fractions: Fraction 1 (**F1**), 0 to 10 min; Fraction 2 (**F2**), 11 to 25 min; Fraction 3 (**F3**), 26 to 35 min; Fraction 4 (**F4**), 36 to 45 min. Norleucine internal standard (.25 μmol) was added to each fraction before lyophilization. Freeze-dried fractions were re-dissolved in 100 μL of .1 M HCl. Free amino acids in F1 and F2 and peptide-bound amino acids (**PBAA**) in all fractions released after acid hydrolysis with 6 M HCl were determined by reversed-phase HPLC as described above.

Calculations and Statistics. Blood flow through the mesenteric-drained viscera (**MDV**) and portal-drained viscera (**PDV**) were calculated as described previously (Seal and Parker, 1994). A positive flux rate indicates apparent release or absorption of a nutrient, whereas a negative flux rate implies apparent uptake or utilization. Statistical analysis of the difference between treatment means for each level of propionic acid was by ANOVA using GLM (Minitab, State College, PA) to examine animal (6 df) and treatment (2 df) effects, weighting steers means to take into account the different number of replicate observations for each steer on the three treatments, using the residual sums of squares as the test term (Seal and Parker, 1994). Treatment sums of squares (2 df) were subdivided into two orthogonal comparisons: 0 vs (.5 + 1.0) and .5 vs 1.0, to compare responses to the presence of propionic acid and level of propionic acid, respectively. Data for mesenteric and portal blood flows and net absorption rates are for five steers, due to losses of either sampling or infusion catheters during the infusion periods. Tables of results show least squares means with residual degrees of freedom for the error term; variables were considered unaffected by propionic acid infusion if $P > .10$.

Results

Metabolite Concentrations and Net Flux

Urea and Ammonia. Despite a small numerical increase in blood urea concentrations as a result of propionic acid infusion (Table 1) there was no effect of propionate supply on arterial or venous concentrations of urea or ammonia. Net metabolism of urea across the MDV and PDV was inconsistent, with a

Table 1. Urea and ammonia concentrations in carotid (C), mesenteric (M), and portal (P) blood, blood flow, and net absorption rates across mesenteric- (MDV) and portal- (PDV) drained viscera in steers fed a grass-pellet diet with intraruminal infusion of propionic acid

Item	Infusion rate, mol/d			EMS ^a	Contrast	
	0	.5	1.0		0 vs .5, 1.0	.5 vs 1.0
Concentration, mM ^b						
Urea						
C	3.20	3.37	3.43	.236	.186	.755
M	3.25	3.46	3.55	.206	.145	.640
P	3.24	3.33	3.48	.254	.345	.503
Ammonia						
C	.097	.093	.114	.0009	.541	.099
M	.220	.213	.216	.0004	.467	.713
P	.244	.225	.234	.0007	.113	.454
Blood flow, L/min ^c						
MDV	2.31	1.94	2.11	.639	.398	.655
PDV	6.08	5.64	5.30	2.241	.341	.665
Net absorption rate, mol/d ^c						
Urea						
MDV	.49	-.08	.29	.306	.127	.811
PDV	.22	-.97	.15	2.078	.247	.167
Ammonia						
MDV	.37	.34	.36	.009	.533	.735
PDV	1.26	1.16	1.02	.064	.136	.319

^aError mean square.

^bn = 7 for C and n = 6 for M and P.

^cMDV: n = 5 for 0 and .5 infusion rates and n = 4 for 1.0 infusion rate. PDV: n = 5 for 0 and .5 infusion rates and n = 3 for 1.0 infusion rate.

small net extraction of the nutrient across the gut tissues in steers receiving .5 mol propionic acid/d in contrast to net appearance during the other experimental periods (Table 1). Net ammonia appearance across the MDV averaged 31% of portal flux and was unaffected by propionate supply (Table 1).

Plasma Free Amino Acids. The concentration of individual plasma-free amino acids is shown in Table 2. Arterial concentrations of several amino acids were greater in steers receiving propionic acid ($P < .1$ for SER, GLN, HIS, CIT, THR, TYR, MET, TRP, ORN, and LYS [Table 2]). Similarly, mesenteric and portal venous concentrations of several amino acids were elevated during propionic acid infusion and, although the trends were the same for both vessels (Table 2), the increase in concentration was greater for mesenteric venous samples ($P < .1$ for SER, ASN, GLY, HIS, ALA, ORN, and LYS) than those from portal plasma ($P < .1$ for ASP, GLY, CIT, THR, VAL, MET, ILE, LEU, TRP, and LYS). Shown cumulatively in Table 3, total amino acid, essential (EAA), and nonessential (NEAA) amino acid concentrations were all elevated in arterial plasma by infusion of propionic acid ($P = .019$; $P = .042$, and $P = .063$, respectively) but were not increased by doubling propionate supply to 1 mol/d. In contrast, mesenteric and portal venous plasma total, EAA, NEAA, and branched-chain (BCAA) amino acid concentrations were all increased during propionate infusion ($P < .1$; Table 3). Portal venous total, EAA

and BCAA, and mesenteric venous BCAA concentrations were further increased at the highest infusion rate ($P < .1$; Table 3). With the exception of GLN during control and .5 mol/d treatments, venous-arterial concentration differences for all the individual amino acids were positive, indicating net appearance of amino acids across the gastrointestinal tract. Net flux of all individual amino acids, except ASP, across MDV was greater in steers that received 1.0 mol propionate/d than in those that received .5 mol propionate/d ($P < .1$; Table 4). Similarly, net flux of SER, ASN, HIS, CIT, TYR, TRP, ORN, and LYS was increased at the highest infusion rate ($P < .1$; Table 4). Net flux of total, EAA, NEAA, and BCAA across MDV was greater for 1.0 compared with .5 mol propionate/d ($P < .03$; Table 5) and was greater for total and EAA across PDV ($P < .1$; Table 5).

Peptide-Bound Amino Acids. A sample chromatogram of low-molecular-weight peptides (<1,500 Da molecular weight) separated by reversed-phase HPLC is shown in Figure 1. The behavior of complex mixtures of peptides during HPLC separation precludes accurate characterization of the peptide fractions; however, approximate sizes for each fraction were determined with reference to peptides of known composition as described previously (Seal and Parker, 1991b). Compared with the concentration of FAA described above, recovery of FAA eluting in F1 and F2 averaged 94.5% (n = 18, SEM 2.24). Recovery of

Table 2. Free amino acid concentration in carotid (C), mesenteric (M), and portal (P) plasma of steers fed a grass-pellet diet with intraruminal infusion of propionic acid

Plasma concentration, μM	Infusion rate, mol/d						Plasma concentration, μM						Infusion rate, mol/d						Contrast													
	0		.5		1.0		0		.5		1.0		0		.5		1.0		0 vs .5, 1.0		.5 vs 1.0		0 vs .5, 1.0		.5 vs 1.0							
	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a	EMS ^a					
ASP																																
C	5	7	6	6	6	6	18	243	.647	.647	.647	.647	143	152	155	155	155	155	545	.168	.796	.168	.796	.168	.796	545	.168	.796	.168	.796	.168	.796
M	13	15	16	16	16	16	15	.167	.659	.659	.659	.659	295	302	364	364	364	364	3,324	.118	.041	.118	.041	.118	.041	3,324	.118	.041	.118	.041	.118	.041
P	6	7	9	9	9	9	5	.095	.300	.300	.300	.300	178	185	207	207	207	207	801	.114	.109	.114	.109	.114	.109	801	.114	.109	.114	.109	.114	.109
GLU																																
C	68	73	76	76	76	76	251	.198	.737	.737	.737	.737	58	62	60	60	60	60	184	.534	.672	.534	.672	.534	.672	184	.534	.672	.534	.672	.534	.672
M	92	95	104	104	104	104	336	.276	.336	.336	.336	.336	133	136	164	164	164	164	644	.114	.038	.114	.038	.114	.038	644	.114	.038	.114	.038	.114	.038
P	73	76	82	82	82	82	171	.249	.342	.342	.342	.342	71	72	83	83	83	83	125	.162	.067	.162	.067	.162	.067	125	.162	.067	.162	.067	.162	.067
SER																																
C	62	72	68	68	68	68	149	.052	.484	.484	.484	.484	51	56	60	60	60	60	62	.016	.231	.016	.231	.016	.231	62	.016	.231	.016	.231	.016	.231
M	195	206	246	246	246	246	1,850	.083	.077	.077	.077	.077	140	143	177	177	177	177	1,079	.142	.047	.142	.047	.142	.047	1,079	.142	.047	.142	.047	.142	.047
P	88	92	104	104	104	104	667	.309	.308	.308	.308	.308	70	72	83	83	83	83	264	.209	.157	.209	.157	.209	.157	264	.209	.157	.209	.157	.209	.157
ASN																																
C	22	29	25	25	25	25	108	.125	.327	.327	.327	.327	398	421	421	421	421	421	2,791	.188	.999	.188	.999	.188	.999	2,791	.188	.999	.188	.999	.188	.999
M	95	103	129	129	129	129	545	.039	.039	.039	.039	.039	583	588	668	668	668	668	5,890	.156	.047	.156	.047	.156	.047	5,890	.156	.047	.156	.047	.156	.047
P	38	42	53	53	53	53	247	.103	.148	.148	.148	.148	436	456	486	486	486	486	1,853	.048	.151	.048	.151	.048	.151	1,853	.048	.151	.048	.151	.048	.151
GLY																																
C	210	223	218	218	218	218	2,276	.482	.792	.792	.792	.792	17	20	19	19	19	19	11	.078	.548	.078	.548	.078	.548	11	.078	.548	.078	.548	.078	.548
M	396	442	482	482	482	482	2,414	.006	.103	.103	.103	.103	68	67	87	87	87	87	358	.243	.045	.243	.045	.243	.045	358	.243	.045	.243	.045	.243	.045
P	253	289	306	306	306	306	4,271	.084	.572	.572	.572	.572	26	30	34	34	34	34	52	.063	.267	.063	.267	.063	.267	52	.063	.267	.063	.267	.063	.267
GLN																																
C	126	137	145	145	145	145	539	.062	.436	.436	.436	.436	164	172	180	180	180	180	488	.163	.343	.163	.343	.163	.343	488	.163	.343	.163	.343	.163	.343
M	158	168	200	200	200	200	1,740	.129	.125	.125	.125	.125	314	314	376	376	376	376	2,909	.173	.030	.173	.030	.173	.030	2,909	.173	.030	.173	.030	.173	.030
P	132	134	162	162	162	162	889	.166	.067	.067	.067	.067	198	205	132	132	132	132	315	.014	.012	.014	.012	.014	.012	315	.014	.012	.014	.012	.014	.012
TAU																																
C	32	33	35	35	35	35	147	.565	.578	.578	.578	.578	213	225	229	229	229	229	792	.134	.737	.134	.737	.134	.737	792	.134	.737	.134	.737	.134	.737
M	39	38	42	42	42	42	77	.776	.421	.421	.421	.421	420	420	498	498	498	498	4,574	.165	.031	.165	.031	.165	.031	4,574	.165	.031	.165	.031	.165	.031
P	37	38	38	38	38	38	213	.765	.967	.967	.967	.967	257	267	293	293	293	293	158	.001	.002	.001	.002	.001	.002	158	.001	.002	.001	.002	.001	.002
HIS																																
C	44	53	47	47	47	47	44	.015	.030	.030	.030	.030	72	75	75	75	75	75	79	.339	.899	.339	.899	.339	.899	79	.339	.899	.339	.899	.339	.899
M	90	93	109	109	109	109	186	.069	.032	.032	.032	.032	171	172	204	204	204	204	994	.195	.048	.195	.048	.195	.048	994	.195	.048	.195	.048	.195	.048
P	54	61	64	64	64	64	223	.112	.647	.647	.647	.647	93	95	106	106	106	106	469	.371	.280	.371	.280	.371	.280	469	.371	.280	.371	.280	.371	.280
CIT																																
C	53	64	66	66	66	66	247	.029	.758	.758	.758	.758	36	40	38	38	38	38	23	.043	.173	.043	.173	.043	.173	23	.043	.173	.043	.173	.043	.173
M	96	107	118	118	118	118	773	.141	.393	.393	.393	.393	67	69	80	80	80	80	109	.111	.046	.111	.046	.111	.046	109	.111	.046	.111	.046	.111	.046
P	63	79	82	82	82	82	428	.038	.792	.792	.792	.792	42	45	48	48	48	48	47	.080	.322	.080	.322	.080	.322	47	.080	.322	.080	.322	.080	.322
THR																																
C	83	96	97	97	97	97	235	.018	.855	.855	.855	.855	77	83	89	89	89	89	180	.057	.278	.057	.278	.057	.278	180	.057	.278	.057	.278	.057	.278
M	207	214	265	265	265	265	2,760	.135	.062	.062	.062	.062	103	106	127	127	127	127	157	.022	.006	.022	.006	.022	.006	157	.022	.006	.022	.006	.022	.006
P	107	112	139	139	139	139	539	.057	.030	.030	.030	.030	86	90	95	95	95	95	445	.384	.636	.384	.636	.384	.636	445	.384	.636	.384	.636	.384	.636
ALA																																
C	175	196	181	181	181	181	1,942	.363	.422	.422	.422	.422	126	139	137	137	137	137	326	.064	.780	.064	.780	.064	.780	326	.064	.780	.064	.780	.064	.780
M	496	499	615	615	615	615	6,561	.084	.012	.012	.012	.012	312	324	390	390	390	390	3,608	.080	.038	.080	.038	.080	.038	3,608	.080	.038	.080	.038	.080	.038
P	247	252	289	289	289	289	3,639	.323	.202	.202	.202	.202	165	180	200	200	200	200	662	.024	.106	.024	.106	.024	.106	662	.024	.106	.024	.106	.024	.106

^aError mean square.^bn = 7 for C and n = 6 for M and P.

Table 3. Total essential (EAA), nonessential (NEAA), and branched-chain (BCAA) amino acid concentration in carotid (C), mesenteric (M), and portal (P) plasma of steers fed a dried grass-pellet diet with intraruminal infusion of propionic acid

Plasma concentration, μM^b	Infusion rate, mol/d			EMS ^a	Contrast	
	0	.5	1.0		0 vs .5, 1.0	.5 vs 1.0
Total						
C	2,235	2,428	2,427	44,370	.019	.985
M	4,484	4,621	5,459	457,011	.012	.179
P	2,719	2,879	3,229	100,373	.017	.037
EAA						
C	1,297	1,393	1,397	18,439	.042	.935
M	2,527	2,567	3,040	163,352	.028	.112
P	1,556	1,635	1,831	13,742	.003	.005
NEAA						
C	938	1,035	1,029	21,277	.063	.917
M	1,957	2,060	2,419	88,172	.006	.715
P	1,168	1,281	1,417	39,652	.031	.164
BCAA						
C	776	818	830	10,093	.157	.757
M	1,317	1,322	1,543	36,563	.059	.067
P	891	928	1,020	2,847	.002	.004

^aError mean square.

^bn = 7 for C and n = 6 for M and P.

PBAA based on hydrolysis of unfractionated filtrate was lower and averaged 91.3% (n = 18, SEM 2.54), suggesting either loss of amino acids during hydrolysis, "leakiness" of individual filters, or larger peptides eluting after 45 min, which were not collected during the isolation procedure. There was no effect of propionic acid infusion on the concentration of individual amino acids in each fraction. Summed across all four fractions, the total PBAA concentration of plasma was unaffected by propionate infusion and averaged 851, 1,935, and 1,610 μM for carotid arterial, mesenteric, and portal venous blood, respectively. Averaged across vessels and treatments the values for each fraction are shown in Figure 2. The PBAA content of F1, F2, and F4 was numerically greater for animals receiving propionic acid, but was lower for F3 ($P = .073$ for total, and $P = .036$ for NEAA, Figure 2). Net PBAA appearance across the MDV and PDV for each fraction and total PBAA is shown in Table 6 and was not affected by supply of propionic acid. In contrast to free amino acids (Tables 4 and 5), net appearance of PBAA across the PDV was greater than across the MDV for each fraction except F2 in control steers.

Discussion

The aim of this experiment was to investigate the possible sparing effect of ruminal propionic acid on glucose, VFA, and amino acid metabolism of the gastrointestinal tract. Data presented in the companion paper (Seal and Parker, 1994) showed that

intraruminal propionic acid infusion resulted in an increase in whole-body glucose turnover and glucose utilization by the portal-drained viscera was reduced. Increased propionic acid supply had little effect on the proportion of the measured ruminal propionate irreversible loss rate metabolized during passage through the rumen wall (51% on the control diet, and 56% on the highest rate of infusion), although quantitatively this would result in more propionate carbon entering metabolic pathways within the tissues. The metabolism of acetate by post-stomach tissues also declined during the infusion period, indicating that manipulation of ruminal conditions had an effect on metabolism of other tissues in the gastrointestinal tract. In addition to glucose, the metabolism of amino acids by the PDV tissues represents a significant cost to the animal in order to maintain the high rates of protein turnover and energetic requirements of the gastrointestinal tissues (Lobley et al., 1980).

The effects of diet composition, in particular protein and energy content, on net flux of amino acids and α -amino-N have been studied in several experiments with sheep and cattle (Seal and Reynolds, 1993). Many of these studies have confirmed the early observations of Tagari and Bergman (1978) that there is a quantitative imbalance between amino acids apparently disappearing from the gut lumen and those subsequently appearing in portal blood. In the present experiment approximately 3.85 mol/d of N was apparently disappearing from the intestine as amino acid-N (calculations based on measurements made on this diet in sheep [Seal et al., 1993a] with amino acids

Table 4. Net amino acid absorption rate across mesenteric- (MDV) and portal- (PDV) drained viscera in steers fed a grass-pellet diet with intramural infusion of propionic acid

Net absorption rate, mol/d ^b	Infusion rate, mol/d						Contrast								
	0			1.0			0 vs .5, 1.0			EMS ^a			0 vs .5, 1.0		
	.5	.0	.5	.5	.0	.5	.5	.0	.5	.5	.0	.5	.0	.5	.0
ASP	.022	.018	.025	.0001	.999	.103	.307	.247	.407	.0147	.627	.023			
MDV	.012	.014	.011	.0001	.964	.521	.215	.137	.223	.0103	.339	.136			
PDV															
GLU															
MDV	.042	.035	.060	.0004	.489	.027	.157	.129	.217	.0038	.454	.017			
PDV	.045	.059	.058	.0023	.505	.972	.085	.073	.121	.0040	.709	.174			
SER															
MDV	.276	.224	.363	.0123	.652	.031	.194	.153	.246	.0056	.799	.031			
PDV	.185	.104	.193	.0081	.268	.093	.128	.081	.119	.0050	.300	.327			
ASN															
MDV	.158	.121	.209	.0037	.689	.016	.381	.292	.498	.0152	.634	.008			
PDV	.105	.067	.135	.0039	.731	.067	.197	.189	.301	.0102	.318	.067			
GLY															
MDV	.367	.322	.489	.0287	.524	.066	.104	.076	.138	.0019	.763	.017			
PDV	.299	.294	.453	.0547	.485	.220	.058	.055	.074	.0019	.741	.395			
GLN															
MDV	.061	.042	.109	.0012	.250	.004	.314	.242	.398	.0132	.818	.020			
PDV	-.004	-.032	.046	.0070	.845	.108	.188	.188	.227	.0102	.671	.464			
TAU															
MDV	.008	.009	.019	.0001	.055	.026	.438	.339	.550	.0231	.847	.019			
PDV	.026	.028	.034	.0022	.815	.796	.266	.236	.278	.0031	.607	.173			
HIS															
MDV	.085	.062	.113	.0010	.732	.009	.209	.167	.264	.0061	.386	.028			
PDV	.065	.044	.093	.0022	.805	.074	.146	.097	.151	.0092	.510	.310			
CIT															
MDV	.093	.062	.099	.0019	.551	.099	.064	.049	.084	.0007	.794	.023			
PDV	.045	.047	.095	.0168	.653	.479	.038	.025	.049	.0003	.627	.031			
THR															
MDV	.252	.192	.334	.0123	.741	.027	.051	.044	.070	.0003	.325	.015			
PDV	.135	.084	.222	.0192	.858	.089	.035	.036	.072	.0007	.146	.033			
ALA															
MDV	.687	.559	.900	.0843	.661	.037	.388	.308	.510	.0261	.682	.029			
PDV	.466	.306	.481	.0463	.346	.151	.260	.229	.332	.0112	.729	.096			

^aError mean square.

^bMDV: n = 5 for 0 and .5 infusion rates and n = 4 for 1.0 infusion rate. PDV: n = 5 for 0 and .5 infusion rates and n = 3 for 1.0 infusion rate.

Table 5. Net total, essential (EAA), nonessential (NEAA), and branched-chain (BCAA) amino acid absorption rate (mol/d) across mesenteric- (MDV) and portal- (PDV) drained viscera in steers fed a grass-pellet diet with intraruminal infusion of propionic acid

Net absorption rate, mol/d ^b	Infusion rate, mol/d			EMS ^a	Contrast	
	0	.5	1.0		0 vs .5, 1.0	.5 vs 1.0
Total						
MDV	4.662	3.694	6.107	2.978	.665	.018
PDV	2.983	2.446	3.726	1.694	.958	.094
EAA						
MDV	2.545	1.976	3.298	.887	.735	.018
PDV	1.564	1.272	1.952	.291	.971	.044
NEAA						
MDV	2.117	1.718	2.808	.661	.592	.022
PDV	1.455	1.505	1.938	.477	.391	.252
BCAA						
MDV	1.134	.873	1.448	.151	.788	.014
PDV	.651	.613	.806	.057	.618	.152

^aError mean square.

^bMDV: n = 5 for 0 and .5 infusion rates and n = 4 for 1.0 infusion rate. PDV: n = 5 for 0 and .5 infusion rates and n = 3 for 1.0 infusion rate.

contributing 70% of absorbed N). The PDV flux of free amino acids represented 77%, 62%, and 97% of this calculated disappearance for control, .5, and 1.0 mol/d, respectively. These values are at the upper end of those reported by Tagari and Bergman (1978) and are greater than the value of 52% obtained from the recovery of [¹³C]leucine infused directly into the duodenum of sheep fed the same diet (Piccioli Cappelli et al., 1993). However, this latter figure may underestimate true flux of leucine if sequestration of arterial ¹³C tracer by the gut tissues is taken into account (MacRae et al., 1993). In contrast to the high values for apparent recovery of amino acid N in the present experiment and those for labeled free amino acids, Guerino et al. (1991) reported that only 28% of casein N infused into the abomasum was detected as α -amino N in the portal vein. Further calculations on available data for PDV flux of FAA confirm the variability in apparent uptake across these tissues (in sheep, 61% to 66% [Piccioli Cappelli, Seal, and Parker, unpublished data] and in steers, 24% to 100% [Seal et al., 1992, 1993b]).

A comparison of net AA uptake across the MDV and the PDV (Table 5) indicates that at the site of sampling the mesenteric blood, net flux of FAA is consistently higher than similar measurements made in the portal vein. In this experiment PDV net flux represents some 65% of MDV flux, and this was not affected by treatment. Data reported by Reynolds and Huntington (1988) in which α -amino-N flux was measured indicated that overall PDV flux was 82% of MDV net flux, although this figure seemed to be higher in forage-fed than in concentrate-fed animals. These values may reflect differences in net utilization of amino acids by stomach tissues (Reynolds et al., 1994) compared with the small intestine. It is also

apparent that net flux rates of FAA measured at sites other than the pars hepatis will be critically dependent on the site of the sampling catheter tip. High rates of net FAA uptake measured in MDV samples in both studies could reflect localized sites of FAA absorption, whereas the PDV values measure overall net uptake across the gut. It is of interest to note that the effect of propionate infusion on the pattern of net uptake of FAA at these two sites is different (Table 5); the 1.0 mol/d intraruminal infusion increased net total FAA uptake across the whole spectrum (i.e., EAA, NEAA, and BCAA) in the MDV, whereas this effect was limited to EAA in the portal vein. This would suggest that venous blood from the stomach tissues, and also that from the large intestine, may have a relatively lower ratio of NEAA and BCAA to EAA compared with blood draining the MDV. This may reflect the reduced demand for amino acids by these sections of the gut, which have been shown in sheep to have a lower protein fractional synthesis rate (Lobley et al., 1995).

Glutamine is a major energy substrate for the gastrointestinal mucosa (Windmeuller and Spaeth, 1978, 1980; Fleming et al., 1991), and net extraction of this amino acid has been reported in several studies with ruminants (Heitmann and Bergman, 1978; Huntington and Prior, 1985; Reynolds and Huntington, 1988). However, in some studies, net appearance of glutamine has also been observed (Prior et al., 1981; Reynolds and Huntington, 1988; Reynolds et al., 1988, 1991; Seal et al., 1992; Balcells et al., 1995). Similar effects are shown in the present experiment, in which net extraction of glutamine by the PDV for control and .5 mol propionate/d treatments was in contrast to net appearance at the highest infusion rate. Altered metabolism of this amino acid measured

by arterio-venous techniques has been linked to changes in diet (Reynolds and Huntington, 1988; Seal et al., 1992), physiological state (Reynolds et al., 1988), and glucose supply (Balcells et al., 1995). These observations have been confirmed *in vitro* by Okine et al. (1995), who demonstrated an effect of stage of lactation on both glutamine and glucose metabolism in enterocytes isolated from dairy cows. Changes in ammonia flux that would be associated with decreased glutaminolysis (Okine et al., 1995) were not observed in our steers; MDV ammonia flux accounted for 31% of PDV flux and neither was affected by treatment (Table 1). The relationship between glutamine metabolism, ammonia flux, and the supply of energy-yielding substrates is unclear from this study.

Blood urea concentrations and net urea fluxes across MDV and PDV were also unaffected by treatment (Table 1). Small arterio-venous concentration differences, and both net appearance and disappearance of urea across the MDV and PDV, are consistent with other experiments in our laboratory (Balcells et al., 1995; Piccioli Cappelli, Seal, and Parker, unpublished data). This is in contrast to many studies that demonstrate uptake of urea by the gut tissues. It is unclear whether this difference is due to the use of enzymatic methods for urea determination compared with colorimetric techniques. Unidirectional flux measurements with labeled urea are needed to resolve this problem.

To quantify N transactions across both MDV and PDV more completely the present study also undertook the analysis of net flux of peptide-bound amino acids using the fractionation method developed at Newcastle (Seal and Parker, 1991b). This process separates a low-molecular-weight (<10,000 Da) peptide fraction by filtration and then HPLC to separate four fractions containing peptides with molecular weights of <1,500 Da. Increased concentration of peptide-N across the gut (Koeln and Webb, 1982; Koeln et al., 1993; Webb et al., 1993) together with decreased amounts across muscle (Danilson et al., 1987) and apparent utilization by the mammary gland (Backwell et al., 1994) has focused attention on the role of these moieties in the transfer of amino acids between tissues. In the present study, in which four peptide fractions were analyzed in mesenteric, portal venous, and carotid arterial blood from steers on the control and 1.0 mol/d intraruminal propionate infusion, there was a net appearance of peptide-N across both the MDV and PDV with no effect of treatment. In contrast to the FAA data, however, there was an increased net appearance into portal blood compared with mesenteric blood, indicating that, if absorbed from the gut, a site of absorption could be the stomach tissues. Hydrolysis of dietary and microbial protein within the rumen results in the transient increase in peptide content of the rumen

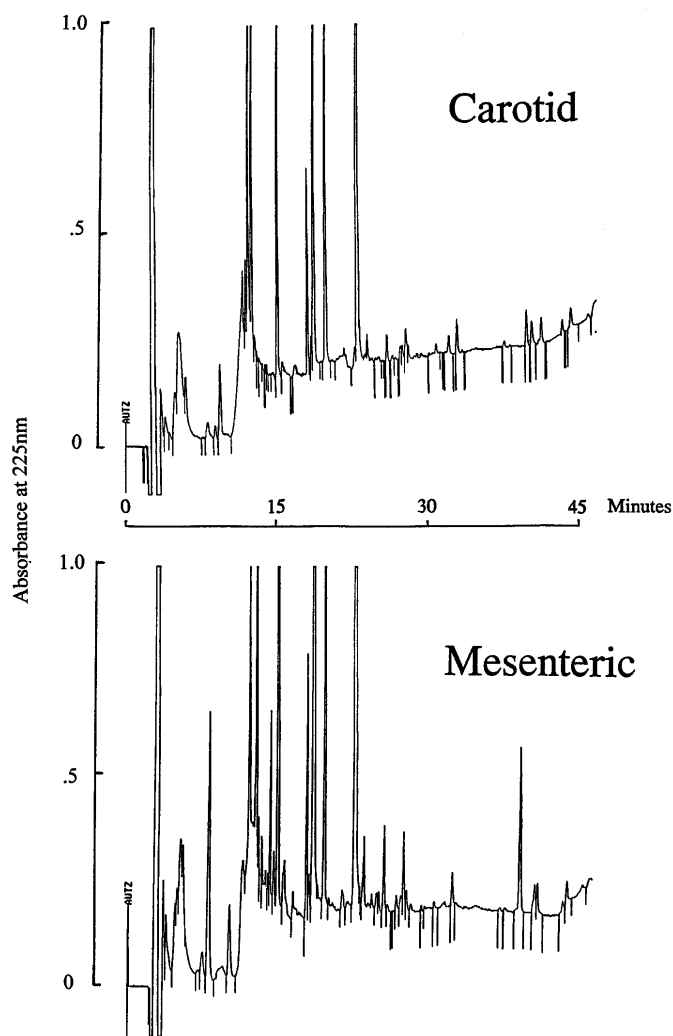


Figure 1. High-performance liquid chromatography separation of low-molecular-weight peptides isolated from carotid arterial and mesenteric venous plasma. Peptides detected by absorbance at 225 nm.

(Wallace and McKain, 1989; Chen and Russell, 1991) as the rate of production exceeds uptake and metabolism by microorganisms. In addition, work by Wallace et al. (1993) identified the presence of degradation-resistant peptides in the ruminal fluid of sheep 6 h after feeding, which were characterized by containing the amino acids aspartate, glycine, and proline. The extent to which peptides accumulate has also been shown to be dependent on protein source and feeding frequency (Chen et al., 1987; Williams and Cockburn, 1991) and, in more recent studies, the interaction between feed protein and physical form of the dietary carbohydrate has been studied (Mesgaran and Parker, unpublished data). Although previous work has focused on the potential contribution of ruminal peptides to N flow in the small intestine (Chen et al., 1987; Broderick and Wallace, 1988), net flux data reported in the present study would support

Table 6. Net total, essential, nonessential, and branched-chain peptide-bound amino acid (PBAA) absorption rate across mesenteric- (MDV) and portal- (PDV) drained viscera in steers (n = 3) fed a grass-pellet diet with intraruminal infusion of propionic acid

PBAA net absorption rate, mol/d	Infusion rate (mol/d)		EMS ^a	<i>P</i> ^b
	0	1.0		
Fraction 1				
Total				
MDV	.386	.525	.502	.832
PDV	.934	1.513	.971	.547
EAA				
MDV	.471	.369	.048	.627
PDV	.606	.863	.103	.429
NEAA				
MDV	-.085	.156	.339	.663
PDV	.328	.649	.443	.615
BCAA				
MDV	.156	.118	.005	.576
PDV	.251	.127	.018	.377
Fraction 2				
Total				
MDV	1.011	1.101	.338	.868
PDV	.631	2.932	8.988	.446
EAA				
MDV	.702	.706	.151	.991
PDV	.255	1.453	1.610	.367
NEAA				
MDV	.309	.394	.041	.657
PDV	.376	1.479	3.002	.517
BCAA				
MDV	.583	.622	.075	.876
PDV	.189	1.288	.908	.293
Fraction 3				
Total				
MDV	.512	.284	.363	.688
PDV	1.322	.169	1.295	.341
EAA				
MDV	.121	.277	.078	.563
PDV	.358	.084	.110	.419
NEAA				
MDV	.391	.007	.149	.348
PDV	.963	.085	.667	.318
BCAA				
MDV	.062	.234	.059	.476
PDV	.142	.038	.022	.482
Fraction 4				
Total				
MDV	.157	.316	.206	.712
PDV	2.149	.325	4.575	.406
EAA				
MDV	.041	.129	.052	.681
PDV	.849	.006	.615	.319
NEAA				
MDV	.117	.186	.052	.746
PDV	1.300	.319	1.920	.477
BCAA				
MDV	.039	.075	.005	.604
PDV	.355	.019	.132	.375
Fraction 1-4				
Total				
MDV	2.067	2.225	.265	.743
PDV	5.037	4.941	1.551	.933
EAA				
MDV	1.335	1.482	.073	.573
PDV	2.068	2.407	.454	.600
NEAA				
MDV	.732	.743	.350	.984
PDV	2.969	2.533	.328	.450
BCAA				
MDV	.385	.525	.502	.832
PDV	.934	1.513	.941	.547

^aError mean square.^bProbability of treatment effect.

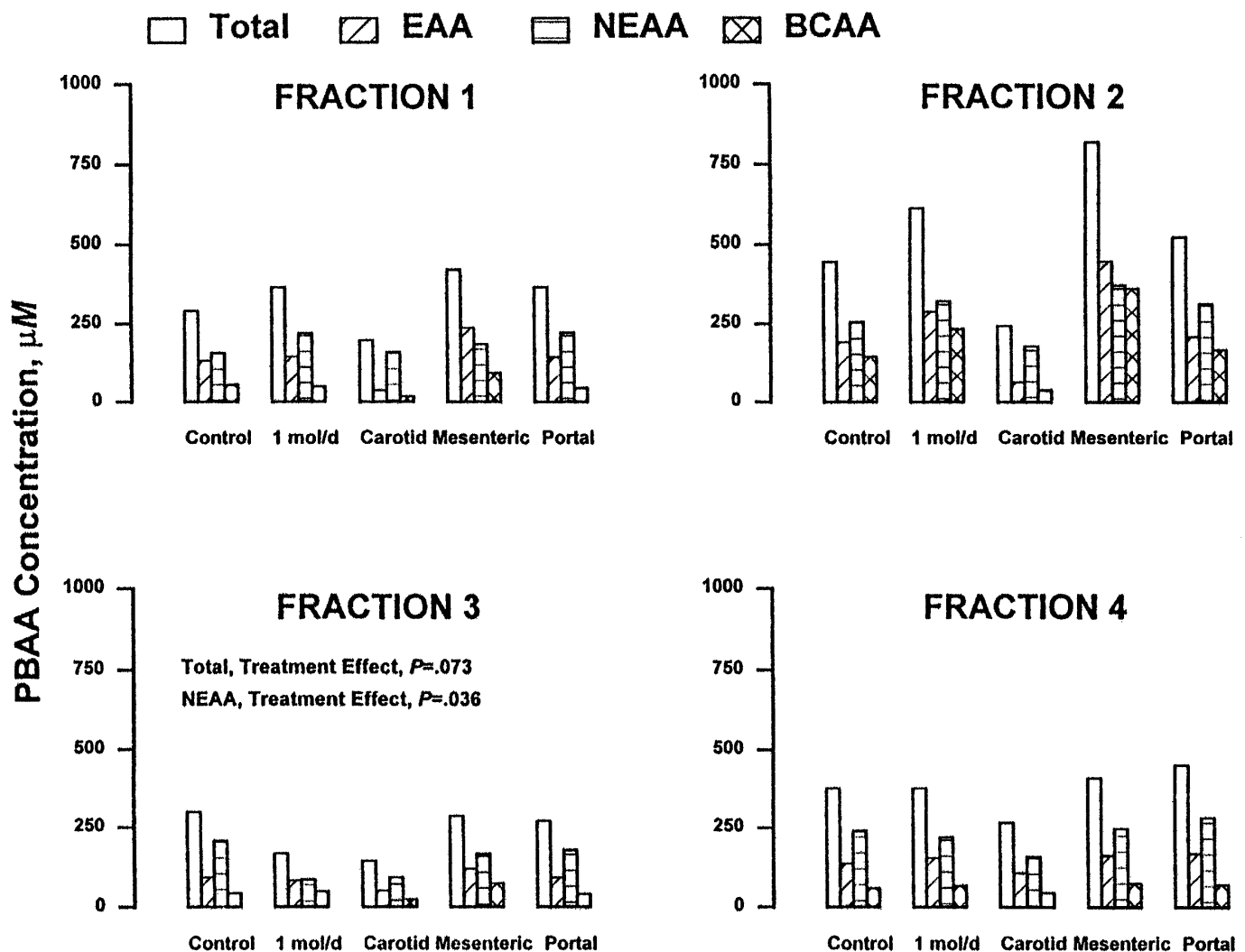


Figure 2. Peptide-bound amino acid concentrations (μM), averaged across vessels for control and 1 mol/d, and averaged across treatments for carotid arterial, mesenteric, and portal venous plasma, in fractions isolated by HPLC. Fraction 1, 0 to 10 min; Fraction 2, 11 to 25 min; Fraction 3, 26 to 35 min, and Fraction 4, 36 to 45 min.

the *in vitro* experiments using rumen and omasal preparations (Webb et al., 1993) identifying the stomach tissues as a major site of peptide uptake in ruminants.

Although there has been extensive debate about the importance of peptide uptake across the gut, there is now clear evidence that a proton-coupled peptide transporter does exist in epithelial tissues on both the apical and basolateral membranes (Ganapathy and Leibach, 1985; Thwaites et al., 1993; Fei et al., 1994). This indicates that even if small peptides absorbed from the gut lumen are extensively hydrolyzed within the cell (Alpers, 1986; Webb, 1990) other peptide fractions resulting from tissue protein turnover could be translocated to the venous blood and contribute to a net efflux of peptide-N from the gut. Peptide transport mechanisms have been identified in liver (Lombardo et al., 1988; Fei et al., 1994), kidney, and brain (Fei et al., 1994) in addition to the intestine, underlining

the potential for tissue utilization of peptide-N as demonstrated in ruminant muscle (Danilson et al., 1987) and mammary gland (Backwell et al., 1994). In the present study analysis of the amino acid content of the peptides present in each fraction isolated demonstrated that although there was no effect of treatment on the net flux rates across the MDV and PDV there were differences in the amino acids present in the peptide fractions from different sampling sites. These are summarized in Table 6 and Figure 2. In both F1 and F2 (quantitatively the major peptide fraction) the predominant component of the peptides analyzed in mesenteric venous samples was EAA, whereas in portal and carotid blood, NEAA was the major contributing group. In F3 and F4 the amino acid composition of the peptides isolated was in the order NEAA > EAA > BCAA for all sites sampled. Changes in the composition of the peptide fractions across different tissue beds may reflect tissue-specific

mechanisms for metabolism of these compounds. The potential for manipulating peptide supply from the gut to match tissue requirements has yet to be elucidated.

Implications

The results presented here, together with those of the companion paper (Seal and Parker, 1994) clearly demonstrate that ruminal volatile fatty acid pattern influences the metabolism of energy-yielding substrates and N-containing fractions across small intestinal and stomach tissues. The mechanism(s) by which these processes occur is unclear, but it seems to represent an integrated response along the gut triggered by events in the rumen. The contribution of low-molecular-weight peptides to the total α -amino-N flux across the gut is considerable. The source of these peptides, their composition, rates of appearance, and the tissues in which they are generated may present possible mechanisms by which N metabolism in ruminants can be manipulated.

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