Nutrient net absorption across the portal-drained viscera of forage-fed beef steers: Quantitative assessment and application to a nutritional prediction model

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Nutrient net absorption across the portal-drained viscera of forage-fed beef steers: Quantitative assessment and application to a nutritional prediction model[1]

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ABSTRACT: This study aimed to establish the relationship between ME intake and energy and nutrient absorption across the portal-drained viscera (PDV) of forage-fed beef steers. Eight Angus (328 ± 40 kg of BW) steers were surgically fitted with portal, mesenteric arterial, and mesenteric venous catheters, and were fed alfalfa cubes in a replicated 4 × 4 Latin square design with 4 levels of energy intake between 1 and 2 times maintenance energy requirements. On d 28 of each experimental period, *p*-aminohippuric acid was infused to measure blood and plasma flow across the PDV, and blood samples (1 every hour, for 6 h) were collected simultaneously from arterial and venous catheters for net absorption measurements. Oxygen utilization, and therefore energy utilization, increased ($P < 0.05$) linearly in relation to ME intake. Glucose net uptake was unaffected, but lactate net release increased linearly in response to ME intake ($P < 0.05$). Net absorption of all AA except tryptophan, glutamate, and glutamine increased linearly with ME intake ($P < 0.05$). The constant net absorption of glutamate and glutamine indicated increased net utilization of these AA when dietary supply was increased. These data provide quantitative measures of the PDV effects on energy and AA availability for productive tissues, and suggest that the greater net utilization of some AA when ME intake is increased could relate to their catabolism for energy production. Prediction estimates of small intestinal AA absorption, based on the Cornell Net Carbohydrate and Protein System (CNCPS), exceeded observed net AA PDV absorption. Mean bias represented the greatest proportion (87 to 96%) of the deviation between individual AA absorption and observed net AA PDV absorption, suggesting that the CNCPS model may be used to predict AA net absorption when factors describing AA utilization by the PDV are applied to model predictions.

Key words: amino acid, beef, energy, intake, metabolism, portal-drained viscera

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INTRODUCTION

Grazing ruminants have ad libitum access to forage; however, environmental conditions, stocking densities, and forage quality have marked effects on voluntary intake. In addition to changes in nutrient supply to the small intestine (Ludden and Kerley, 1997; Elizalde et al., 1999b), previous studies have shown that feed intake also influences gastrointestinal tract (**GIT**) mass (Burrin et al., 1990; McLeod and Baldwin, 2000). Such changes affect our ability to predict nutrient availability to postabsorptive tissues.

Feeding graded levels of a single diet is a means to study changes in metabolism caused by increased supply of nutrients to the GIT with minor effects on the profile of AA flowing to and subsequently absorbed from the small intestine (Ludden and Kerley, 1997; Elizalde et al., 1999b). To date, most existing data regarding ME intake and its effect on energy metabolism and nutrient absorption by the portal-drained viscera (**PDV**) of cattle stem from studies in which 2 ME levels of the same diet were fed (Reynolds et al., 1991a,b; Reynolds et al., 1992). In studies where more than 2 levels of ME intake were used, feeding has been limited to mixed

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Table 1. Nutrient composition of the alfalfa cubes fed to steers at 4 levels of ME intake[1]

Component, analyzed[2]	% of DM
NDF	42.3
ADF	30.5
CP	19.7
Ash	10.4
Water-soluble carbohydrates	8.7
Lignin	7.5
Crude fat	2.2
Starch	1.5
ME,[3] Mcal/kg	2.18

[1]Top dressed with 45 g/d of a mineral premix that contained per kilogram: 600 g of salt; 387 g of ammonium chloride; 5,791 mg of iron; 3,434 mg of zinc; 2,991 mg of manganese; 72 mg of iodine; 41 mg of cobalt; 11 mg of selenium.

[2]Chemical analyses conducted by near infrared reflectance spectroscopy according to AOAC method 989.03 (AOAC, 2005).

[3]DE calculated according to NRC (2001), and ME calculated from DE, using the equation ME = 0.82 × DE (NRC, 2000).

high-concentrate diets (Huntington and Prior, 1983; Lapierre et al., 2000). However, differences in tissue mass were reported when sheep were on high-forage compared with high-concentrate diets, even when ME intake was constant (McLeod and Baldwin, 2000). For that reason, the response of the PDV to high-concentrate diets cannot appropriately predict nutrient absorption when animals consume forages.

The objective of the current study was to determine how increments of alfalfa intake, and, thus, increments of ME intake, affect nutrient and energy net absorption across the PDV of beef cattle and to establish response relationships between intake and nutrient net availability to postabsorptive tissues. These data would be useful to incorporate into nutritional models to improve prediction of growth performance for cattle consuming forage.

MATERIALS AND METHODS

All surgical and experimental protocols were approved by the University of Kentucky Animal Care and Use Committee (protocol # 387A2002).

Steers and Surgery

Eight Angus steers (328 ± 40 kg of BW) were fitted with portal, mesenteric arterial, and mesenteric venous catheters according to procedures adapted from Huntington et al. (1989). Before surgery, steers were deprived of feed and water for 24 and 12 h, respectively. Immediately before surgery, animals were fitted with a temporary jugular catheter to facilitate pre- and postoperative antibiotic treatment regimens. On the day of and for 3 d following surgery, animals were administered ceftiofur sodium (2.2 mg/kg of BW), penicillin G potassium (6,000,000 units), and flunixin meglumine (2.2 mg/kg of BW). Animals were initially anesthetized by i.v. injection of xylazine hydrochloride (0.09 mg/kg of BW) and ketamine hydrochloride (1.8 mg/kg of BW), intubated, and maintained with isoflurane (3 to 5%) in O_2. Following placement and exteriorization over the paralumbar shelf and spine, catheters were filled with heparinized saline solution (1,000 units/mL) containing penicillin G potassium (6,000 units/mL). A recovery period of at least 2 wk preceded initiation of experimental treatments, and during this time, health status was monitored daily via rectal temperature and feed intake.

Steers were housed in individual stalls with ad libitum access to water in an environmentally controlled room. Ambient temperature was maintained at 23.8°C with a 16-h light cycle. Following surgery, steers were offered alfalfa cubes at approximately 1.5% of BW daily, and twice-daily intake allotments were gradually increased as normal, healthy bowel activity and appetite were observed.

Treatments and Sampling

Experimental design was a replicated 4 × 4 Latin square balanced for residual effects. Treatments consisted of 4 equally spaced levels of dietary intake (alfalfa cubes; Table 1), designed to provide ME ranging from 0.117 to 0.234 Mcal of ME/(kg of $BW^{0.75}$·d), which approximated 1 to 2 times ME requirements (NRC, 2000). Experimental periods lasted 28 d, and through d 5 steers were gradually adapted to their respective intakes. Through d 19, daily feed allotments were offered twice daily at 0730 and 1500 h, and the amount of feed offered was adjusted once weekly based on BW measurements obtained every 7 d. Beginning on d 20 and continuing throughout the sample collection period, feed was offered in 12 equal portions delivered every 2 h using automatic feeders (Ankom, Fairport, NY). Orts, when present, were collected daily before the morning feeding, weighed, and sampled for DM determination to calculate daily DMI.

Blood samples were obtained on d 27 or 28, and steers were randomly selected for sampling day each period. Beginning at 0800 h, arterial and portal blood samples (2 samples of each; 25 and 1 mL) were simultaneously collected into heparinized syringes at hourly intervals for 6 h. The 25-mL samples were transferred to 50-mL conical bottom centrifuge tubes, and the 1-mL samples were maintained in their respective syringes. All samples were placed on ice, and transported to the laboratory for processing and analysis.

Blood and plasma flows across the PDV tissue bed were determined by downstream dilution of a continuous infusion (1.0 g/min; Model 205U cassette pump, Watson-Marlow Brendel Pumps Inc., Wilmington, MA) of 250 m*M* *p*-aminohippurate (**PAH**; pH 7.4) into the mesenteric venous catheter. Infusion of PAH (0700 to

1400 h) began 1 h before the first blood sample collection. The PAH was prepared using sterile water, filtered through sterile 0.45-μm cellulose acetate bottle-top filters (Corning, Corning, NY), and autoclaved. From the reservoir, PAH was pumped through sterile 0.22-μm syringe filters (Millipore, Bedford, MA) and sterilized tubing into sterilized 500-mL bottles, one for each steer.

Sample Analyses

In the laboratory, the 1-mL blood samples were immediately analyzed for O_2 saturation and hemoglobin (Hemoximeter, model OSM2, Radiometer America, Westlake, OH). The 25-mL blood samples were subsampled, and 1 mL of blood was diluted (1:3 wt/wt) with deionized water and analyzed for PAH (Harvey and Brothers, 1962) and urea-N (Marsh et al., 1965) by automated colorimetric procedures (AAII, Technicon Instruments Corp., Tarrytown, NY). The remaining blood was centrifuged (3,000 × *g*, 20 min) and the plasma harvested within 30 min of blood sampling. Plasma samples were analyzed for glucose and lactate (model 2300, Yellow Springs Instrument, Yellow Springs, OH); then, 1 mL of plasma was diluted (1:3 wt/wt) with deionized water and PAH was measured as before. The remaining plasma was stored frozen (−80°C) until further analyses could be performed.

Plasma AA concentrations were determined by isotope dilution gas chromatography-mass spectrometry as described previously (Calder et al., 1999; El-Kadi et al., 2006). Fresh plasma samples (0.5 g) were spiked with an equal weight of an internal standard solution containing 0.2 mg of hydrolyzed [U-^{13}C]algae protein powder (99 atom percent; Martek Biosciences Corp., Columbia, MD), 100 nmol of [indole-$^{2}H_5$]tryptophan, 200 nmol of [5-^{15}N]glutamine, and 25 nmol of [methyl-$^{2}H_3$]methionine, and samples were frozen (−80°C) until further analysis. Thawed samples were deproteinized by the addition of 1 mL of sulfosalicylic acid (15% wt/vol), centrifuged (13,000 × *g* at 4°C), and the supernatant was desalted by cation (AG-50, H^+ form) exchange by washing twice with 2 mL of water. Amino acids were eluted with 1 mL of 2 *M* NH_4OH followed by 1 mL of water, and the eluates were freeze-dried. Amino acids were transferred to v-vials (Pierce, Rockford, IL) with 200 μL of 0.1 *N* HCl, dried under a stream of N_2, and converted to their *t*-butyldimethylsilyl derivatives before gas chromatography-mass spectrometry (HP 5973N Mass Selective Detector, Agilent, Palo Alto, CA). Mass spectrometry was conducted under electron impact mode, and the following ions (*m*/*z*) were monitored: alanine 260, 263; glycine 246, 248; valine 288, 293; leucine 302, 308; isoleucine 302, 308; proline 286, 291; methionine 292, 295; serine 390, 393; threonine 404, 408; phenylalanine 234, 242; aspartate 302, 304; glutamate 432, 437; lysine 300, 306; glutamine 431, 432; histidine 440, 446; tyrosine 302, 304; and tryptophan 375, 380.

Calculations

Plasma and whole blood flow rates across the PDV were calculated using the Fick principle (Katz and Bergman, 1969): plasma/blood flow (L/h) = $IR_{PAH}/(Cv_{PAH} - Ca_{PAH})$, where IR_{PAH} is PAH infusion rate (μmol/h), and Cv_{PAH} and Ca_{PAH} are PAH concentrations (μ*M*) in venous and arterial blood, respectively. Net absorptions of nutrients across the PDV were computed as follows: Net absorption = (BF or PF) × (Cp – Ca), where Ca and Cp are nutrient concentrations in arterial and portal blood, and BF and PF are blood or plasma flow, respectively. A positive net absorption indicated absorption or release of a nutrient, and a negative net absorption denoted uptake or utilization by the PDV.

Whole-blood oxygen concentration was calculated (Huntington and Tyrrell, 1985) using the following equation: O_2 (m*M*) = Hgb × 1.34 × % O_2 × (1/22.4), where Hgb is blood hemoglobin (g/L), 1.34 is mL of O_2/g of hemoglobin, % O_2 is the percentage O_2 saturation, and 22.4 is O_2 volume at standard temperature and pressure (mL of O_2/mmol of O_2). The oxygen extraction ratio was calculated as $(A_{O_2} - V_{O_2})/A_{O_2}$, where A_{O_2} and V_{O_2} are arterial and venous oxygen concentrations (Jones et al., 1989). Heat production was calculated as 4.89 kcal/L of O_2 consumed (Huntington and Tyrrell, 1985).

Model Inputs

Diet composition has been reported to influence prediction models, and a greater degree of accuracy would be attained by using the chemical composition of the offered diet rather than using model libraries (Alderman et al., 2001; Bateman et al., 2001; Fox et al., 2004). Therefore, individual animal information for BW, feed intake, and diet composition (Table 1; Dairy One Inc., Ithaca, NY) were used in the Cornell Net Carbohydrate and Protein System (**CNCPS**; v. 5.0.40) to predict net absorption of individual essential AA (**EAA**). When parameter information was not available, the default conditions of the model were used.

Statistical Analyses

Means were calculated within steer and period for arterial and portal concentrations of glucose, lactate, blood urea, AA, and PAH. Daily means were used to calculate venous-arterial differences, and net absorptions were obtained from the product of venous-arterial differences and the mean blood or plasma flow. An ANOVA was conducted using PROC MIXED (SAS Inst. Inc., Cary, NC). Data were analyzed as a replicated 4

Table 2. Body weight and daily ME intake and DMI for each dietary intake level

	ME intake, Mcal/(kg of $BW^{0.75}$·d)					
Item	0.117	0.156	0.195	0.234	SEM	*P*-value
BW, kg	328	328	332	327	26	0.85
ME intake, Mcal/(kg of $BW^{0.75}$·d)	0.117[a]	0.155[b]	0.187[bc]	0.194[c]	0.0099	<0.001
DMI, kg/d	4.15[a]	5.51[b]	6.74[bc]	6.89[c]	0.63	<0.001

[a–c]Within a row, means without a common superscript letter differ ($P < 0.05$).

× 4 Latin square design, balanced for residual effects (Cochran and Cox, 1992). The statistical model included the fixed effect of treatment and the random effects of square, steer, and period. The following linear mixed model was used:

$$Y_{ijkl} = \mu + T_i + R_j + C_k + S_l + \varepsilon_{ijkl},$$

where Y_{ijkl} is the observed value for the lth square, kth steer, the jth period and the ith treatment, μ is the grand mean, and ε_{ijkl} is the random error associated with Y_{ijkl}. When a significant feed intake effect was detected, means were separated using Tukey-Kramer multiple comparison test. A backward stepwise regression was performed, where a third-order model was tested, and if not significant, the analysis was repeated with a lower order model (Draper and Smith, 1981).

To evaluate how well model predictions fit observed data, mean square prediction errors (**MSPE**) were calculated (Theil, 1966; Bibby and Toutenburg, 1977):

$$MSPE = \frac{1}{n}\sum_{i=1}^{n}(P_i - O_i)^2, \quad [1]$$

where P_i is the predicted AA absorption from the digestive tract (CNCPS), O_i the observed AA PDV net absorption, and n is the number of observations. The MSPE for each individual AA was decomposed into error terms associated with mean bias, linear bias, and residual error (Theil, 1966; Bibby and Toutenburg, 1977). Mean bias, which represents the average inaccuracy of the model (Kohn et al., 1998), was calculated thus:

$$\text{Mean bias} = \frac{1}{n}\sum_{i=1}^{n}(P_i - O_i). \quad [2]$$

Linear bias was the slope of the regression line of residuals (predicted minus observed) vs. predicted AA net absorption, and the residual error was calculated as the remaining error after accounting for mean and linear biases (Kohn et al., 1998). Bias was considered significant when mean bias or slope of the regression lines were different from zero ($P < 0.05$).

The CNCPS-predicted absorption for individual AA was regressed on measured PDV net absorption. The relationship between predicted absorption and net absorption across the PDV yields the apparent net absorption efficiency (slope of regression line) for each AA, and the 95% confidence intervals were constructed to determine whether the slopes of individual EAA differed from unity (El-Kadi et al., 2006).

RESULTS

The portal blood flow of one animal showed a large variation within 2 blood collection days, possibly a result of improper PAH mixing, which may have been caused by laminar flow in the portal vein (Burrin et al., 1989). Therefore, data from this animal were treated as missing for these 2 d. One animal from the 0.195 and 3 animals from the 0.234 Mcal of ME/(kg of $BW^{0.75}$·d) treatments did not consume all of their allotted feed. However, corrections for ME were performed based on feed consumption and all data were used to calculate the regression parameters (Table 2).

Increasing dietary ME intake (Table 3) linearly increased arterial and portal blood urea ($P < 0.05$). This simultaneous increase in blood urea in arterial and portal blood resulted in no change in net removal by the PDV. Averaged across treatments, urea was transferred on a net basis to the PDV at a rate of 19.0 mmol/h. In contrast, ME intake had no effect on glucose or lactate arterial and portal plasma concentrations. Glucose net uptake by the PDV was also unaffected by ME intake (30 mmol/h); nonetheless, lactate net release by the PDV increased in response to ME intake ($P = 0.006$).

Arterial and portal blood oxygen concentrations significantly decreased ($P < 0.05$) when ME intake was increased from 0.117 to 0.234 Mcal of ME/(kg of $BW^{0.75}$·d). This coincided with an increase in oxygen utilization ($P < 0.001$), and therefore equated to an increase (2.321 to 3.834 Mcal/d) in energy utilization by the PDV ($P < 0.001$). Despite the increased blood flow to the PDV, the oxygen extraction ratio also increased as ME intake increased ($P = 0.02$).

Increasing ME intake linearly increased ($P < 0.05$) arterial plasma concentrations (Table 4) of most EAA, with the exception of lysine and histidine. However, for the nonessential AA, only arterial proline concentrations increased with increased intake ($P = 0.006$). Portal plasma concentration (Table 5) of all AA except

Table 3. Linear regression of arterial and portal blood nutrients (m*M*) and their portal-drained viscera (PDV) net absorptions (mmol/h), on ME intake [Mcal/(kg of $BW^{0.75}$·d)] of steers fed increments of ME intake[1]

Item	Intercept (*a*)	SE_a	Slope (*b*)	SE_b	*P*-value[2]	R^2
Arterial concentration, m*M*						
Blood						
Urea	2.9	0.35	4.9	1.8	0.02	0.77
Oxygen	6.9	0.38	−5.4	1.9	0.01	0.79
Plasma						
Glucose	3.7	0.33	3.4	2.0	0.09	0.43
Lactate	0.9	0.20	−1.2	1.2	0.32	0.22
Portal concentration, m*M*						
Blood						
Urea	2.5	0.43	6.7	2.4	0.01	0.73
Oxygen	5.4	0.36	−6.0	1.9	0.006	0.79
Plasma						
Glucose	3.6	0.33	3.0	2.0	0.15	0.42
Lactate	1.0	0.21	−1.0	1.2	0.41	0.24
Portal blood flow, L/h	107	120	3395	670	<0.001	0.62
Portal plasma flow, L/h	74	88	2043	490	<0.001	0.58
PDV net absorption, mmol/h						
Blood						
Urea	15	15	−135	90	0.14	0.73
Oxygen	−147	220	−5,481	920	<0.001	0.75
Oxygen extraction ratio	0.20	0.027	0.41	0.16	0.02	0.54
PDV heat production, Mcal/d	0.4	0.65	16.4	3.6	<0.001	0.81
Plasma						
Glucose	1	19	−165	114	0.16	0.07
Lactate	17	17	234	76	0.006	0.66

[1]SE_a = SE of the intercept (*a*) estimate; SE_b = SE of the slope (*b*) estimate.
[2]Probability that the slope estimate = 0.

histidine, glycine, aspartate, glutamate, and glutamine increased linearly with intake ($P < 0.05$).

For all AA, except for tryptophan, glutamate, and glutamine, the relationship between AA net absorption across the PDV and ME intake was significant (Table 6), where PDV net absorption increased ($P \leq 0.004$) in response to ME intake. The negative glutamine net absorption across the PDV for all intake levels indicated a net removal of this AA at all ME intake levels.

The CNCPS mean small intestinal absorption exceeded ($P < 0.05$) mean PDV net absorption by 6 to 41 μmol/(kg of BW·h) for all EAA (Table 7). Mean bias was

Table 4. Linear regression of arterial AA concentration (μmol/kg) on energy intake [Mcal/(kg of $BW^{0.75}$·d)] of steers fed increments of ME intake[1]

AA	Intercept (*a*)	SE_a	Slope (*b*)	SE_b	*P*-value[2]	R^2
Histidine	44	4.0	−27	23	0.25	0.54
Isoleucine	13	13	311	73	<0.001	0.60
Leucine	38	17	320	98	0.003	0.41
Lysine	38	10	106	57	0.08	0.57
Methionine	2	2.2	51	11	<0.001	0.75
Phenylalanine	23	4.9	96	28	0.002	0.53
Threonine	16	7.9	127	44	0.01	0.62
Tryptophan	11	3.9	49	21	0.03	0.56
Valine	77	24	549	140	<0.001	0.52
Alanine	98	13	103	67	0.14	0.42
Aspartate	5	0.71	−4	4.2	0.37	0.36
Glutamate	40	11	−30	64	0.64	0.14
Glutamine	130	17	63	90	0.49	0.57
Glycine	180	19	−123	98	0.22	0.47
Proline	21	3.8	59	19	0.006	0.69
Serine	34	5.6	47	32	0.15	0.31

[1]SE_a = SE of the intercept (*a*) estimate; SE_b = SE of the slope (*b*) estimate.
[2]Probability that the slope estimate = 0.

Table 5. Linear regression of portal AA concentration (μmol/kg) on energy intake [Mcal/(kg of $BW^{0.75}$·d)] of steers fed increments of ME intake[1]

AA	Intercept (*a*)	SE_a	Slope (*b*)	SE_b	*P*-value[2]	R^2
Histidine	42	4.6	4	27	0.87	0.62
Isoleucine	5	15	404	82	<0.001	0.63
Leucine	25	19	469	110	<0.001	0.51
Lysine	33	13	196	70	0.01	0.63
Methionine	−1	3.1	90	17	<0.001	0.76
Phenylalanine	19	7.0	170	43	0.001	0.46
Threonine	8	10	210	56	0.001	0.68
Tryptophan	9	3.8	65	21	0.006	0.72
Valine	65	26	680	150	<0.001	0.55
Alanine	89	15	263	78	0.003	0.74
Aspartate	5	1.1	2	6.1	0.75	0.33
Glutamate	49	12	−78	74	0.30	0.12
Glutamine	113	18	102	100	0.32	0.58
Glycine	190	20	−92	110	0.43	0.43
Proline	18	4.9	105	25	<0.001	0.76
Serine	31	8.8	132	49	0.01	0.50

[1]SE_a = SE of the intercept (*a*) estimate; SE_b = SE of the slope (*b*) estimate.
[2]Probability that the slope estimate = 0.

the largest contributor (86 to 96%) to MSPE, and therefore to prediction accuracy. Slope bias was significantly different from zero for all AA, indicating larger overprediction (slope > 0) when small intestinal supply of digested AA was increased. Residual error ranged from 4 to 12% of MSPE.

The net portal recovery of absorbed EAA (Table 8), except tryptophan, increased linearly with increased small intestinal AA absorption ($P < 0.001$). The net absorption efficiency (slope of the regression line) ranged from 0.40 for tryptophan to 0.88 for methionine. The slopes of all AA, except for methionine, were all <1 (95% CI) indicating their net utilization by the PDV.

DISCUSSION

Nutrient availability to postabsorptive tissues has been shown to be affected by PDV nutrient utilization, not only during absorption (i.e., first pass), but also upon being presented again (i.e., second pass) to PDV tissues from the systemic circulation (MacRae et

Table 6. Linear regression of portal-drained viscera AA net absorption (mmol/h) on energy intake [Mcal/(kg $BW^{0.75}$·d)] of steers fed increments of ME intake[1]

AA	Intercept (*a*)	SE_a	Slope (*b*)	SE_b	*P*-value[2]	R^2
Histidine	−1.1	0.35	13	1.7	<0.001	0.82
Isoleucine	−4	1.3	40	7.7	<0.001	0.61
Leucine	−6	1.9	57	11	<0.001	0.56
Lysine	−4	1.5	50	8.8	<0.001	0.66
Methionine	−2.0	0.54	20	3.1	<0.001	0.57
Phenylalanine	−3	1.1	39	6.4	<0.001	0.67
Threonine	−5	1.5	42	8.6	<0.001	0.58
Tryptophan	−0.6	0.68	6	4.1	0.18	0.43
Valine	−5	1.7	45	9.5	<0.001	0.59
Alanine	−11	3.6	113	21	<0.001	0.64
Aspartate	−0.2	0.19	4	1.1	0.001	0.50
Glutamate	2	2.1	−9	13	0.49	0.02
Glutamine	−3	1.9	−7	11	0.52	0.52
Glycine	−1	2.2	39	12	0.004	0.69
Proline	−2.3	0.75	24	4.3	<0.001	0.65
Serine	−5	1.8	55	10	<0.001	0.34

[1]SE_a = SE of the intercept (*a*) estimate; SE_b, SE of the slope (*b*) estimate.
[2]Probability that the slope estimate = 0.

Table 7. Mean small intestinal AA absorption predicted by the Cornell Net Carbohydrate and Protein System [μmol/(kg of BW·h)] compared with observed mean AA net absorption across the portal drained viscera [μmol/(kg of BW·h)]

	Predicted		Observed						Percentage of MSPE[1]		
AA	Small intestinal absorption	SEM	PDV net absorption	SEM	RMSPE[2]	Mean bias	Slope bias	Residual error	Mean bias	Slope bias	Residual error
	μmol/(kg of BW·h)								%		
Histidine	13.9	0.65	3.0	0.44	11.2	11.0*	0.56*	2.2	95.8	0.25	4.0
Isoleucine	36.7	1.7	7.2	1.3	30.2	29.5*	0.50*	6.4	95.5	0.03	4.5
Leucine	50.6	2.4	10.4	1.7	41.2	40.2*	0.50*	8.8	95.4	0.01	4.6
Lysine	41.0	1.9	10.8	1.5	31.0	30.2*	0.45*	6.8	95.2	0.02	4.8
Methionine	10.6	0.46	3.5	0.57	7.3	7.1*	0.12*	2.0	92.5	0.03	7.5
Phenylalanine	27.5	1.3	8.5	1.1	19.6	19.0*	0.37*	4.5	94.6	0.04	5.4
Threonine	36.6	1.7	6.4	1.3	30.9	30.2*	0.48*	6.5	95.5	0.02	4.4
Tryptophan	6.8	0.32	1.3	0.39	5.9	5.5*	0.60*	2.1	86.6	1.03	12.4
Valine	45.3	2.1	7.2	1.6	38.9	38.1*	0.54*	8.2	95.5	0.02	4.4

[1]MSPE = mean square prediction error.
[2]RMSPE = root mean square prediction error, calculated as √MSPE; n = 30.
*Bias differed from 0 ($P < 0.05$).

al., 1997a). Therefore, PDV metabolism plays a role in determining the efficiency at which nutrients are absorbed and utilized. Because of the positive relationship between forage intake and GIT mass (McLeod and Baldwin, 2000), this role is even more important in animals consuming forage diets because of the physiological changes that occur in the GIT under these management conditions. Data on nutrient net absorption across the PDV over a range of forage ME intakes are limited. We set out to determine how ME intake may affect nutrient delivery to postabsorptive tissues. The goal of this study was to provide data for incorporation into nutritional models for accurately predicting nutrient delivery to postabsorptive tissues and, hence, predicting animal growth performance.

The current data indicate that glucose net uptake by the PDV occurred at a rate independent of ME intake, and that lactate appearance in the portal blood increased as ME intake increased. Furthermore, arterial glucose concentration was unaffected by ME intake however blood flow increased. Therefore, even when systemic glucose supply was increased, visceral tissue glucose net uptake remained constant. Similar results were reported for heifers fed 3 levels of a high (75% corn) concentrate diet (Huntington and Prior, 1983). However, others have reported that in steers fed a 75% alfalfa diet, PDV glucose net uptake increased with increased feed intake, and a larger net uptake of glucose coincided with an increase in lactate net release (Reynolds et al., 1991b). Those authors concluded that lactate results from glucose metabolism in the PDV.

Oxidation to CO_2 and metabolism to lactate accounted for most of the glucose uptake and utilization in rumen epithelial and duodenal mucosal cells in vitro (Harmon, 1986; Oba et al., 2004). In those studies, propionate addition also increased lactate production. The contribution of glucose and propionate to lactate net release cannot be established from net absorption measurements. However, given that glucose net absorption across the PDV was constant in our study, it is possible that the increased lactate net release could have resulted from increased propionate metabolism by PDV tissues. Ruminal propionate production has been shown to increase in steers fed alfalfa ad libitum compared with those whose intake was restricted (Elizalde et al., 1999a). Changes in the contribution of glucose and propionate to lactate net release warrant further examination.

The PDV net absorption of all AA except tryptophan, glutamate, and glutamine increased with forage intake. The duodenal flow of tryptophan has been reported to increase in response to feeding graded levels of a high concentrate diet (Ludden and Kerley, 1997). The lack of a significant increase in tryptophan net absorption in the current study may be attributed to its low content in microbial proteins flowing to the duodenum (Ludden and Kerley, 1997).

The negative glutamine net absorption across the PDV occurred at a constant rate with increased ME

Table 8. Linear regression of portal-drained viscera net absorption of AA [μmol/(kg of BW·h)] on small intestinal absorption of AA predicted by the Cornell Net Carbohydrate and Protein System [μmol/(kg of BW·h)] for steers fed increments of ME intake[1]

AA	Intercept (*a*)	SE_a	Slope (*b*)	SE_b	*P*-value[2]	R^2	95% confidence interval for *b*
Histidine	−3	1.1	0.44	0.057	<0.001	0.83	0.33, 0.56
Isoleucine	−11	3.7	0.50	0.090	<0.001	0.65	0.31, 0.68
Leucine	−15	5.1	0.50	0.093	<0.001	0.60	0.31, 0.69
Lysine	−12	4.2	0.55	0.090	<0.001	0.71	0.36, 0.74
Methionine	−6	1.6	0.88	0.14	<0.001	0.70	0.59, 1.16
Phenylalanine	−9	3.0	0.63	0.10	<0.001	0.68	0.42, 0.83
Threonine	−12	4.0	0.52	0.10	<0.001	0.62	0.31, 0.72
Tryptophan	−1	1.5	0.40	0.21	0.07	0.39	−0.03, 0.83
Valine	−14	4.7	0.46	0.093	<0.001	0.63	0.27, 0.65

[1]SE_a = SE of the intercept (*a*) estimate; SE_b = SE of the slope (*b*) estimate.
[2]Probability that the slope estimate = 0.

intake. Conversely, there was a net appearance of glutamate (positive net absorption) across the PDV, but this appearance was also unaffected by ME intake. Our data are consistent with previous reports in which glutamine and glutamate net absorption across the PDV of cattle occurred at a constant rate even when feed intake (Huntington and Prior, 1983; Reynolds et al., 1992; Lapierre et al., 2000) or intestinal protein supply (Bruckental et al., 1997) increased. Given that the glutamate and glutamine net absorptions were constant despite the increased small intestinal supply (Elizalde et al., 1999b) implies that PDV net utilization was greater as ME intake increased. A direct implication for their increased utilization is that the requirements for glutamate and glutamine by other body tissues may have to be met via de novo synthesis (Reeds et al., 1996).

Glutamine has been considered the preferred energy substrate of small intestinal cells in which oxidation to CO_2 represents up to 50% of small intestinal glutamine utilization in rats (Windmueller and Spaeth, 1974, 1978, 1980). Although this contribution is lower in ruminants, it accounts for 10 to 25% of glutamine utilization in rumen papillae (Harmon, 1986) and isolated small intestinal cells (Okine et al., 1995). Similar data for glutamate are not available for ruminants; however, in pigs, glutamate has been shown to be almost completely catabolized by small intestinal cells during absorption (Reeds et al., 1996). In the current experiment, it is possible that the net utilization of glutamate and glutamine increased because of increased GIT mass (Burrin et al., 1990; McLeod and Baldwin, 2000); hence, an increase in energy requirement occurred to maintain GIT integrity and secretory functions.

Energy utilization by the PDV, measured as oxygen consumption, increased linearly in response to the increase in ME intake and as a result represented approximately 25% of ME intake. Dietary changes have a substantial impact on PDV energy utilization (Reynolds et al., 1991a), and, subsequently, the energy available for productive tissues. Those changes relate to increased nutrient absorption and maintenance of GIT tissues (McBride and Kelly, 1990) that occur because of increased GIT mass in response to ME intake (Burrin et al., 1990; McLeod and Baldwin, 2000).

Using stoichiometric estimations, Baldwin (1995) suggested that energy costs directly associated with nutrient absorption are relatively small compared with costs associated with maintenance of GIT function; that is, protein turnover and ion transport. Although the protein content of GIT tissues is unaffected by feed intake (Lobley et al., 1994; McLeod and Baldwin, 2000), increased GIT mass would translate to an increase in protein mass. Similarly, feed intake only moderately influences mass-specific Na^+-K^+-dependent respiration (McLeod and Baldwin, 2000). Therefore, the increased GIT mass would increase the absolute amount of energy required to maintain tissue function, and would explain the increased PDV energy utilization observed in this study.

In ruminants, small intestinal AA supplies are derived from a combination of microbial, feed, and endogenous proteins. The CNCPS includes a submodel that predicts individual AA supply to and absorption from the small intestine, based on small intestinal digestibility of dietary and bacterial protein fractions (Sniffen et al., 1992; O'Connor et al., 1993; Fox et al., 2004). The model also predicts the efficiency of AA utilization for growth and is assumed to be a function of equivalent shrunk body weight (Fox et al., 1992; Ainslie et al., 1993). One limitation of this assumption is that predictions are based on whole-animal response, and therefore individual AA metabolism by absorptive and postabsorptive tissues is not considered (Hanigan et al., 1997; 1998; 2006).

The goal of model evaluation is to identify areas where prediction models need further improvements (Hanigan et al., 2006). It is clear from the current data and others (Hanigan et al., 2004; Pacheco et al., 2006) that not accounting for AA metabolism by the

PDV would result in biased predictions of postabsorptive AA supply (Hanigan et al., 2004). Accordingly, one objective of the current study was to compare CNCPS predictions of AA absorption with actual net absorption data across the PDV of beef steers consuming forage diet. Although the CNCPS model is not intended to predict net PDV absorption (Lapierre et al., 2006; Pacheco et al., 2006), reparameterization may allow for the accurate prediction of net PDV absorption of AA. Thus, the discrepancy between model prediction of AA absorption from the small intestine and measured PDV net absorption could be partitioned into error terms (Bibby and Toutenburg, 1977), and depending on the error, proper model adjustment(s) may be suggested to increase model accuracy (Kohn et al., 1998; Bateman et al., 2001). Because the model predicts AA absorption from the small intestine without accounting for PDV net utilization, the difference between predicted and observed net absorption would represent the proportion of AA used by the PDV (Pacheco et al., 2006).

Mean bias, which measures the average deviation of the predictions from measured values, varied from 6 to 41 μmol/(kg of BW·h) indicating that CNCPS model predictions of AA absorption exceeded measured net absorption values. Despite the significant slope bias of all AA, the contribution to MSPE did not exceed 1.1%. Therefore, the large proportion of MSPE accounted for by mean bias (87 to 96%), and slope bias (up to 1%), conversely the small proportion of residual error, suggests that linear model adjustments could be performed. Similar observations have also been reported in dairy cows, where CNCPS predictions exceeded measured net PDV absorption of EAA (Pacheco et al., 2006). The current data set indicates that CNCPS may be used to predict net PDV AA absorption in forage-fed steers once linear corrections are applied, accounting for both mean and slope bias. However, because we have previously shown that GIT mass, and presumably PDV AA utilization, is influenced not only by ME intake, but also diet composition (McLeod and Baldwin, 2000), it is unlikely that the same linear corrections could be applied across all types of diets.

Assuming that the error involved in predicting absorbed AA supply is small relative to net PDV utilization, the relationship between model prediction and PDV net absorption would indicate the proportion by which EAA are net removed by the PDV. Our data suggest that this relationship was linear for all EAA; therefore, PDV net utilization represented a constant proportion of an increased EAA supply.

It is well recognized that net portal recovery does not account for all small intestinal AA disappearance (MacRae et al., 1997a; Berthiaume et al., 2001) due to PDV metabolism of AA from luminal and arterial supplies (MacRae et al., 1997b). The apparent net absorption efficiency in the current study for all EAA, except methionine, was significantly lower than 100% indicating net utilization by the PDV. These values are lower than what was previously reported in growing sheep (El-Kadi et al., 2006). In that study, however, the increased AA supply to the small intestine was due to casein infusion, whereas in the current study the increased supply was from increased feed intake. Therefore, the lower net absorption efficiency of EAA may relate to an increase in PDV utilization resulting from an increase in GIT mass in response to forage ME intake (McLeod and Baldwin, 2000).

Previous studies have shown that ME intake affects GIT nutrient metabolism. This study provides response relationships between ME intake and PDV energy and nutrient net absorption in steers fed a forage diet. Our data indicated that the increase in forage ME intake caused an increase in AA and energy net removal by the PDV. This means that the PDV would greatly affect energy and AA availability to postabsorptive tissues given the large contribution of the PDV to whole-body oxygen consumption and AA net utilization. Furthermore, prediction models such as the CNPCS could be adjusted to predict net PDV absorption of EAA, which may be used as a better indicator for AA supply to productive tissues. Our data suggest that, in ruminants fed forage diets, applying apparent recoveries of individual EAA to CNCPS predictions give better estimates of AA net absorption across the PDV.

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