# The effect of forage and forage–concentrate diets on rumen fermentation and metabolism of nutrients by the mesenteric- and portal-drained viscera in growing steers

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**Growing Friesian steers chronically catheterized in the anterior mesenteric and portal veins were used to study the influence of feeding with either a forage or forage–concentrate diet on nutrient utilization by mucosal tissue. When animals were consuming the forage–concentrate diet the molar proportion of propionate in rumen fluid was significantly increased, although production rate as measured by isotope dilution was not altered. Net rates of absorption of VFA into portal blood when compared with rumen production rates underlined the extent to which metabolism within mucosal tissue modifies the propionate supply to the liver. Net glucose utilization by splanchnic tissue was shown to be significantly lower on the forage–concentrate diet. There were no effects of diet on whole-body glucose turnover or on the proportion of glucose derived from propionate. Animals fed on the forage–concentrate diet had significantly lower concentrations of circulating essential amino acids, due mainly to a reduction in branched-chain amino acid levels. There was net absorption of all amino acids by animals on both diets except for glutamate, glutamine and taurine in forage-fed animals.**

**Nutrient absorption: Gut tissue metabolism: Amino acid uptake: Steer**

Tissues of the splanchnic bed have a considerable demand for oxygen and maintain a high metabolic activity associated with digestive and absorptive processes (Huntington & Tyrrell, 1985; Reynolds *et al.* 1986). Energy demands by the visceral tissue are dependent on many factors including stage of development, lactation, metabolizable energy intake and level of nutrition (Smith & Baldwin, 1974; Webster *et al.* 1975; Edelstone & Holzman, 1981; Huntington & Prior, 1983; Weighart *et al.* 1986; Burrin *et al.* 1989). Thus, level of intake and starvation have been shown to influence $Na^+/K^+$-transporting ATPase (*EC* 3.6.1.37)-dependent respiration in duodenal tissue of sheep in vitro (McBride & Milligan, 1985; Kelly & McBride, 1990) and, more specifically, infusion of volatile fatty acids (VFA) into the rumen results in proliferation of the mucosal cells (Sakata & Tamata, 1978). Substrates available to the mucosa for oxidative metabolism in addition to arterial glucose supply vary in different regions of the gut and will be dependent on the composition of the diet. The extent to which metabolism of available nutrients by the gut tissues is influenced by diet has been the subject of a number of studies using chronically catheterized animals in which net absorption of metabolites into the portal vein has been measured (Huntington & Prior, 1983; Huntington, 1984; James *et al.* 1985; Reynolds & Huntington, 1988*a*, *b*; Burrin *et al.* 1989). The net absorption of amino acids from the gastrointestinal tract of sheep and cattle has been reported in a number of studies using animals chronically catheterized in the portal vein (Hume *et al.* 1972; Wolff *et al.* 1972; Sniffen & Jacobsen, 1975; Huntington & Prior, 1985). Studies in sheep in which apparent absorption of amino

acids from the small intestine was compared with their appearance in portal blood (Tagari & Bergman, 1978) underline the extent to which metabolism by intestinal tissues may influence amino acid availability to the liver and peripheral tissues. In addition, net portal absorption of amino acids in cattle has been shown to be influenced by both protein content of the diet and energy intake (Sniffen & Jacobsen, 1975; Huntington & Prior, 1985). Recent work reported by Reynolds & Huntington (1988*a*), however, in which net absorption of α-amino-nitrogen into the mesenteric and portal veins was determined, suggest that changes in the metabolism of the mucosa of the small intestine as a result of dietary manipulation may not be detected by measurements made only in the portal vein.

The objective of the present study was to investigate the influence of rumen fermentation on metabolism of energy-yielding metabolites by the gastrointestinal tissues and the net absorption of amino acids and other nutrients into the mesenteric and portal veins of growing steers fed on a forage or forage–concentrate diet. Preliminary results from part of this experiment have been published (Seal *et al.* 1989).

## MATERIALS AND METHODS

### *Animals and diets*

Four Friesian steers weighing between 116 and 137 kg at the start of the experiment were used. Chronic indwelling Silastic catheters (Dow Corning Corporation, Midland, MI, USA) were inserted in the portal vein (1·016 mm i.d., 2·159 mm o.d.), the anterior mesenteric vein caudal to the mesenteric vein–gastrosplenic vein junction (0·762 mm i.d., 1·651 mm o.d.) and a carotid artery (0·762 mm i.d., 1·651 mm o.d.) (Symonds & Baird, 1973). Two smaller infusion catheters (0·635 mm i.d., 1·193 mm o.d.) were implanted in a distal branch of the mesenteric vein. Each animal was also fitted with a small rumen fistula (40 mm i.d.) and all surgery was completed at least 3 weeks before the start of any collections. The animals were housed in individual stalls with *ad lib.* access to drinking water and were fed on either a forage diet (F) of dried-grass pellets or a forage–concentrate diet (F–C) prepared from the same dried grass and flaked maize in the ratio 50:50 (total N (g/kg dry matter (DM)) 31·1, 24·0; neutral-detergent fibre (g/kg DM) 551, 319; gross energy (MJ/kg DM)18·2, 18·3, for diet F and diet F–C respectively). The experiment was of a cross-over design using two pairs of animals with 7 d equilibration between diets. The order in which diets were fed was balanced across the four animals which were fed hourly in twenty-four equal lots from mechanical bucket-type feeders at a fixed metabolizable energy intake calculated from Agricultural Research Council (1984) tables to give a weight gain of 700 g/d. For each dietary treatment whole-body glucose turnover rate was measured by intravenous infusion of [6-$^{3}$H]glucose. Rumen propionate production rate and contribution of propionate to gluconeogenesis was determined on a separate occasion following intraruminal infusion of [2-$^{14}$C]propionic acid. During each of these infusions net nutrient absorption rates and blood flow within the splanchnic bed were also determined.

### *Infusions and sampling procedure*

Blood samples taken from catheters implanted in the portal vein represent venous blood draining the total digestive tract and thus nutrients presented to the liver from the portal-drained viscera (PDV). A part of this blood supply, sampled through the anterior mesenteric vein catheter, arises from the mesenteric-drained viscera (MDV) which represents venous blood draining the splanchnic bed up to the rumen. Contributions by the rumen to net PDV nutrient absorption can therefore be calculated by difference between PDV and MDV absorption rates. In all cases, a negative arterio–venous difference (V–A)

or absorption rate indicates net removal of nutrient from arterial blood by the splanchnic bed; positive V–A or absorption rate indicates net addition of nutrient by the splanchnic bed.

*Blood flow.* Blood flow in the portal vein and anterior mesenteric vein was determined by downstream dye dilution using *p*-amino hippuric acid (PAH; Katz & Bergman, 1969). Blood samples (15 ml) were taken hourly mid-feed from the portal vein, mesenteric vein and carotid artery during the last 6 h of a 7·5 h primed, continuous infusion of PAH into the distal mesenteric vein (800 mg PAH priming dose, 40 mg PAH/ml per min infusion rate) and treated immediately for subsequent metabolite analyses.

*Measurement of whole-body glucose turnover rate.* A solution of [6-$^3$H]glucose (Amersham International plc, Aylesbury, Bucks.) was prepared in autoclaved physiological saline (9 g sodium chloride/l) containing 1·5 $\mu$Ci [6-$^3$H]glucose/ml and 1 mg carrier glucose/ml, and infused for 7·5 h at approximately 1·0 ml/min using a Watson Marlow Model 501 peristaltic pump (Watson Marlow Ltd, Falmouth, Cornwall) via a temporary jugular catheter inserted the previous day. Blood samples (15 ml) taken hourly from the carotid artery into ice-cold heparinized tubes during the last 6 h of the infusion period were immediately centrifuged at 2500 rev/min at 4° (IEC Centra-7R centrifuge; Damon/IEC Division, Needham Hts, MA, USA) and the plasma was frozen at −20° for plasma glucose specific radioactivity determinations.

*Measurement of propionate metabolism.* Steady-state rumen propionate specific activity was established with a 12 h continuous intrarumen infusion of [2-$^{14}$C]propionic acid (Amersham International plc) in saline (0·125 $\mu$Ci/ml per min, 1 mg carrier propionic acid/ml). To ensure adequate mixing in the rumen, the isotope was infused at three sites and rumen fluid was removed from a fourth through a stainless steel filter. Rumen fluid samples (50 ml) were taken hourly during the last 6 h of the infusion. Subsamples were acidified immediately with orthophosphoric acid (200 ml/l) and frozen at −20° until analysed. Carotid blood samples also taken during the last 6 h were treated as previously described.

### *Analytical methods*

Whole-blood glucose concentrations were determined enzymically using a YSI 23A Glucose Analyser (Clandon Scientific Ltd, Aldershot, Hants). PAH in the supernatant fraction from deproteinized whole-blood samples (whole blood–trichloracetic acid (TCA, 100 g/l); 1:12, v/v) was determined colorimetrically (Katz & Bergman, 1969). Urea and ammonia concentrations were measured in the supernatant fraction from whole blood deproteinized with TCA (whole blood–TCA (100 g/l); 1:1, v/v) by a colorimetric method using diacetyl monoxime (Moore & Kauffman, 1970) and enzymically by the method of Bergmeyer & Beutler (1985) respectively. Plasma L-lactate concentrations were determined using L-lactate dehydrogenase (*EC* 1.1.1.27; Boehringer Mannheim test combination; Boehringer Corp. Ltd, Lewes, Sussex). 3-Hydroxybutyrate concentrations were measured in the supernatant fraction from whole blood deproteinized with perchloric acid (PCA; whole blood–0·6 M-PCA, 1:1, v/v) by autoanalyser using 3-hydroxybutyrate dehydrogenase (*EC* 1.1.1.30; Lloyd *et al.* 1978). VFA were determined in the supernatant fraction from whole blood deproteinized with PCA (whole blood–0·6 M-PCA, 1:1, v/v; 2·5 mM-2-methyl butyric acid internal standard). A sample of supernatant fraction (0·5 ml) was neutralized on ice with 2 M-potassium carbonate to remove excess perchlorate. The solution was re-acidified with M-oxalic acid, centrifuged and the supernatant fraction chromatographed by gas–liquid chromatography (GLC) on a Carbopak B-DA, 4% CW 20 M column (Supelco Inc., Belefonte, PA, USA; 4 mm × 2·5 m column, oven temperature 136°, injector/detector temperature 200°, $N_2$ carrier gas flow rate 30 ml/min).

*Rumen fluid analysis.* The VFA content of rumen fluid was determined by GLC using GP 10% SP-1200/1% $H_3PO_4$ on 80/100 Chromosorb W AW (Supelco Inc.). Rumen ammonia-N was determined by steam distillation of rumen fluid preserved by the addition of equal volumes of 0·2 M-hydrochloric acid. Propionate specific activity was determined in propionate eluted from high-performance liquid chromatography (HPLC) fractionation of deproteinized rumen fluid using an Aminex HPX-87H ion-exchange column (4 mm × 250 mm; Bio-Rad Chemical Division, Richmond, CA, USA), at 50° with 5 mM-sulphuric acid at a flow rate of 0·5 ml/min. Fractions (0·5 ml) from the column were mixed with 4·5 ml liquid scintillant (LKB Optiphase 'Hisafe' II; Pharmacia LKB Biotechnology, Milton Keynes, Bucks.) and counted on a Beckman LS 8100 Liquid Scintillation Counter (Beckman Instruments Inc., Irvine, CA, USA).

*Analysis of glucose specific radioactivity.* The level of radioactivity of plasma glucose from both [6-$^3$H]glucose and [2-$^{14}$C]propionate infusions was determined after preparing the glucose penta-acetate derivative (Jones, 1965) and counting by liquid scintillation using LKB Optiphase 'Hisafe' II as scintillant.

*Amino acid analysis.* Plasma samples collected during one 6 h sampling period from each animal on both diets were bulked before analysis. Free amino acids in the supernatant fraction from deproteinized plasma (plasma–0·6 M-PCA, 2:1, v/v; 1·0 mM-α-amino butyric acid as internal standard) were determined by reversed-phase HPLC after pre-column derivatization with phenylisothiocyanate using a Waters Pico-Tag System (Waters Chromatography Division, Millipore Corporation, Milford, MA, USA).

### *Calculations and statistical analysis*

Rumen propionate production rate and whole-body glucose turnover rate were determined by standard isotope techniques (White *et al.* 1969) using the appropriate ratio of isotope infusion rate and plateau propionate or glucose specific activity (SA). No allowance for interconversion of propionate with other VFA was included. The percentage of glucose synthesized from rumen propionate, calculated by the method of Leng *et al.* (1967), reflects transfer of primary substrate to secondary product through all available pathways and therefore does not represent direct transfer of C between the two pools. The rate of blood flow at each sampling catheter tip was calculated as described by Katz & Bergman (1969). Net nutrient absorption rates were calculated as the product of blood flow rate and blood V–A concentration differences for the total PDV and the MDV. No correction for water absorption across the MDV and PDV was made.

Statistical analysis of the difference between means for each dietary treatment was by Student's *t* test using Glim 3.12 (Baker & Nelder, 1978), having allowed for any differences between animals and weighting animal means to take into account the different number of replicate observations for each animal on the two diets. For each 6 h sampling period, values from duplicate samples were averaged to give a single observation for each animal. Replicate observations for metabolite concentrations and net flux taken on different sampling days were averaged for each animal. Period effects were tested for but were found not to be significant; since no long-term responses were expected during the course of the experiment, period effects were therefore not fitted separately in the analysis but were included in the residual error term. As a consequence of the small number of animals available for the study there were some effects due to animal variation but these were inconsistent. Within-animal variation was always less than between-animal variation for replicate observations on each diet. Animal and diet interactions were tested for but were not significant for any variable and have not been reported. Results for nutrient concentrations and net absorption rates (Tables 3 and 4) include all available values from replicate sampling periods within each treatment. These measurements were obtained at

Table 1. *Volatile fatty acid (VFA) and ammonia-nitrogen contents of rumen fluid from steers fed on forage and forage–concentrate diets* and difference between diets for each metabolite*

(Means of replicate observations from four animals; 3 df)

| | Forage† | Forage–concentrate | Difference between diets‡ | *t* | Statistical significance of difference: $P <$ |
|---|---|---|---|---|---|
| Total (mM) | 167·4 | 168·6 | 2·7 | 0·22 | NS |
| VFA (mol/100 mol) | | | | | |
| Acetate | 74·9 | 64·6 | −10·2 | 24·21 | 0·01 |
| Propionate | 16·2 | 22·2 | 6·1 | 7·79 | 0·01 |
| Isobutyrate | 0·4 | 0·9 | 0·6 | 11·76 | 0·01 |
| Butyrate | 7·6 | 9·1 | 1·3 | 0·93 | NS |
| Isovalerate | 0·4 | 1·2 | 0·9 | 9·81 | 0·01 |
| Valerate | 0·6 | 2·1 | 1·6 | 4·78 | 0·05 |
| $NH_3$-N (mg/l) | 185·2 | 90·9 | −95·2 | 7·32 | 0·01 |

NS, not significant (Student's *t* test).

* For details of diet and feeding regimen, see p. 356.

† Mean value of all replicates.

‡ Forage–concentrate − forage; calculated value from weighted means; for details of statistical methods, see pp. 358–359.

least twice on each animal in parallel with estimates of glucose turnover and propionate metabolism, but in some instances were made on further occasions where infusions were repeated. Results are presented as diet means averaged across all animals and replicates with an estimated difference between diets (F–C − F) weighted to take into account the unequal number of replicate observations for each animal. Propionate production rates, glucose kinetics and amino acid results (Tables 2, 5 and 6) are from single measurements made on each animal and diet. In view of the small number of degrees of freedom, significant differences have been quoted up to the 10% level.

## RESULTS

### *Rumen fermentation and propionate production rate*

Details of the pattern of rumen fermentation are shown in Table 1. Although the total VFA content of rumen fluid was unaffected by dietary treatment, the molar proportions of individual VFA were significantly different when animals were fed on diet F compared with diet F–C. The proportions of propionate, isobutyrate, isovalerate and valerate were all significantly greater ($P < 0·01$, $P < 0·05$ for valerate) in diet F–C-fed animals than in diet F-fed animals, and this increase was at the expense of acetate which contributed a lower proportion to the total VFA pool in animals fed on the mixed diet ($P < 0·01$). Despite the proportion of propionate in rumen fluid rising from 0·16 to 0·22 on changing diets from F to F–C, the production rate of this VFA was not significantly affected by diet.

N intakes (g/d) were 96 and 57 for diets F and F–C respectively, and this difference in intake and the form of N available is reflected in the lower concentration of rumen $NH_3$-N for animals fed on diet F–C compared with diet F-fed animals ($P < 0·01$, Table 1).

### *Glucose turnover rate and contribution of propionate to glucose-C*

Whole-body glucose turnover rate, as measured by dilution of infused [6-$^3$H]glucose, averaged 2·46 mmol/min and was higher for animals fed on diet F–C compared with diet

Table 2. *Propionate production rates, glucose kinetics and difference between diets for steers fed on forage and forage–concentrate diets**

(Mean values for four animals; 3 df)

| | Forage | Forage–concentrate | Difference between diets† | *t* | Statistical significance of difference: $P <$ |
|---|---|---|---|---|---|
| Propionate production rate (mmol/min) | 2·95 | 3·30 | 0·35 | 0·83 | NS |
| Whole-body glucose turnover rate (mmol/min) | 2·18 | 2·74 | 0·57 | 2·51 | 0·10 |
| Proportion of glucose derived from propionate | 0·51 | 0·55 | 0·04 | 0·74 | NS |
| Proportion of propionate converted to glucose | 0·76 | 0·91 | 0·14 | 1·34 | NS |

NS, not significant (Student's *t* test).
* For details of diet and feeding regimen, see p. 356.
† Forage–concentrate – forage.

F-fed animals ($P < 0·10$, Table 2). The contribution of propionate to glucose-C calculated from the transfer quotient during [2-$^{14}$C]propionate infusion (Table 2) was unaffected by dietary treatment and showed that approximately 0·53 of whole-body glucose was derived from rumen propionate. The proportion of propionate-C converted to glucose averaged 0·83 across both diets and was not affected by treatment (Table 2).

*Whole-blood metabolite concentrations and net nutrient absorption rates*

Blood flow in the portal and mesenteric veins measured by dilution of PAH is shown in Table 4. On average, mesenteric blood flow represented 0·53 of portal blood flow. There was no significant difference in blood flow in animals fed on either diet F or diet F–C.

*Glucose*

Circulating glucose levels were significantly higher ($P < 0·05$) in animals fed on diet F–C compared with animals fed on diet F (Table 3). Glucose V–A and net absorption rates across the MDV and PDV were negative for animals fed on diet F, indicating net utilization of glucose by the splanchnic tissue. In contrast, V–A for diet F–C-fed animals was positive for MDV and PDV.

*Lactate*

Circulating blood lactate concentrations tended to be lower for animals fed on diet F–C than for diet F-fed animals and were significantly lower in mesenteric and portal blood in animals fed on diet F–C ($P < 0·05$, Table 3). Lactate production across the PDV of diet F–C-fed animals was significantly lower than for the diet F-fed group and, overall, animals fed on diet F–C had an apparent net production of lactate 0·97 mmol/min lower than when fed on diet F alone ($P < 0·05$, Table 4).

Table 3. *Nutrient concentrations (mmol/l) in carotid (C), mesenteric (M) and portal (P) blood of steers fed on forage and forage–concentrate diets* and difference between diets at each sampling site*

(Mean of replicate observations from four animals for C and M (3 df), three animals for P (2 df))

| Metabolite | | Forage† | Forage–concentrate | Difference between diets‡ | *t* | Statistical significance of difference: $P <$ |
|---|---|---|---|---|---|---|
| Glucose | C | 3·808 | 4·244 | 0·468 | 4·50 | 0·05 |
| | M | 3·747 | 4·325 | 0·618 | 7·19 | 0·01 |
| | P | 3·671 | 4·362 | 0·778 | 7·41 | 0·05 |
| Lactate | C | 0·722 | 0·635 | −0·112 | 0·96 | NS |
| | M | 0·796 | 0·694 | −0·122 | 3·39 | 0·05 |
| | P | 0·810 | 0·629 | −0·169 | 5·74 | 0·05 |
| Ammonia | C | 0·213 | 0·172 | −0·039 | 2·54 | 0·10 |
| | M | 0·480 | 0·363 | −0·114 | 4·33 | 0·05 |
| | P | 0·536 | 0·369 | −0·165 | 3·01 | 0·10 |
| Urea | C | 3·53 | 1·71 | −1·77 | 11·27 | 0·01 |
| | M | 3·42 | 1·69 | −1·69 | 11·56 | 0·01 |
| | P | 3·48 | 1·69 | −1·74 | 55·73 | 0·001 |
| Acetate | C | 1·13 | 1·05 | −0·08 | 1·37 | NS |
| | M | 1·19 | 1·21 | −0·05 | 0·18 | NS |
| | P | 2·16 | 1·95 | −0·22 | 1·27 | NS |
| Propionate | C | 0·03 | 0·05 | 0·05 | 1·97 | NS |
| | M | 0·05 | 0·15 | 0·08 | 1·29 | NS |
| | P | 0·20 | 0·27 | 0·07 | 1·91 | NS |
| Butyrate | C | ND | | | | |
| | M | ND | | | | |
| | P | 0·04 | 0·03 | −0·01 | 0·88 | NS |
| 3-OH-But | C | 0·499 | 0·524 | 0·028 | 0·59 | NS |
| | M | 0·550 | 0·507 | −0·062 | 1·17 | NS |
| | P | 0·642 | 0·671 | 0·032 | 0·56 | NS |

NS, not significant (Student's *t* test); ND, not detected; 3-OH-But, 3-hydroxybutyrate.

* For details of diets and feeding regimen, see p. 356.

† Mean value of all replicates.

‡ Forage–concentrate − forage; calculated value from weighted means; for details of statistical methods, see pp. 358–359.

### *$NH_3$ and urea*

Blood ammonia and urea concentrations were significantly higher for animals fed on diet F than when they were fed on diet F–C (Table 3). Net absorption rates of $NH_3$ across the MDV and PDV were positive for all animals on both diets and were significantly higher for diet F-fed animals compared with diet F–C-fed animals ($P < 0{\cdot}10$, Table 4). Urea V–A for MDV and PDV showed extraction of this nutrient by the splanchnic bed and this was not affected by dietary treatment. Net urea absorption rates across the gut tissues were also unaffected by diet.

### *VFA and 3-hydroxybutyrate*

VFA concentrations in carotid, mesenteric and portal venous blood were unaffected by dietary treatment, although acetate absorption across the PDV was significantly lower in diet F–C-fed animals ($P < 0{\cdot}05$, Table 4). Butyrate concentrations in all but portal samples were below reliable detection limits and were unaffected by diet ($P > 0{\cdot}1$). 3-Hydroxybutyrate levels, which showed considerable variation between animals, V–A

Table 4. *Portal and mesenteric blood flow (l/min) and net nutrient absorption rates in mesenteric-drained viscera and portal-drained viscera and difference between diets for steers fed on forage and forage–concentrate diets**

(Mean for replicate observations for three animals; 2 df)

| | Forage† | Forage–concentrate | Difference between diets‡ | *t* | Statistical significance of difference: $P <$ |
|---|---|---|---|---|---|
| Blood flow (l/min) | | | | | |
| Mesenteric | 2·806 | 2·529 | −0·286 | 0·66 | NS |
| Portal | 5·203 | 4·946 | −0·263 | 1·04 | NS |
| Mesenteric absorption rate (mmol/min) | | | | | |
| Glucose | −0·159 | 0·265 | 0·429 | 2·87 | NS |
| Lactate | 0·017 | −0·149 | −0·085 | 0·21 | NS |
| Ammonia | 0·599 | 0·399 | −0·178 | 3·55 | 0·10 |
| Urea | −0·057 | 0·135 | 0·234 | 0·63 | NS |
| Acetate | −0·213 | 0·267 | 0·724 | 1·35 | NS |
| Propionate | −0·028 | 0·275 | 0·289 | 0·86 | NS |
| Butyrate | — | — | — | — | — |
| 3-OH-But | 0·076 | −0·015 | −0·073 | 1·18 | NS |
| Portal absorption rate (mmol/min) | | | | | |
| Glucose | −0·315 | 0·281 | 0·815 | 1·34 | NS |
| Lactate | 0·984 | 0·052 | −0·966 | 5·09 | 0·05 |
| Ammonia | 1·602 | 0·965 | −0·745 | 3·29 | 0·10 |
| Urea | −0·751 | −0·111 | 0·806 | 0·84 | NS |
| Acetate | 5·179 | 4·787 | −0·379 | 6·11 | 0·05 |
| Propionate | 0·872 | 0·982 | 0·045 | 1·66 | NS |
| Butyrate | 0·216 | 0·171 | −0·047 | 1·29 | NS |
| 3-OH-But | 0·602 | 0·641 | −0·029 | 0·25 | NS |

NS, not significant (Student's *t* test); 3-OH-But, 3-hydroxybutyrate.

* For details of diets and feeding regimen, see p. 356.

† Mean value of all replicates.

‡ Forage–concentrate − forage; calculated value from weighted means; for details of statistical methods, see pp. 358–359.

differences and absorption rates for MDV and PDV were similarly unaffected by dietary treatment.

*Amino acids*

The concentrations of individual plasma free amino acids (FAA) are shown in Table 5. In peripheral blood samples from animals fed on diet F there were significantly higher concentrations of valine, methionine and isoleucine ($P < 0·05$) and leucine, tryptophan and lysine ($P < 0·10$) than those obtained when diet F–C was given. Similarly, in mesenteric blood the concentration of branched-chain amino acids (BCAA) was significantly higher when animals were fed on diet F (valine, isoleucine and leucine, $P < 0·05$). In portal blood sampled from animals on the two diets, only glycine and proline showed any difference between the two treatments; in this case the concentration of these two amino acids was significantly lower ($P < 0·10$) when diet F was given compared with diet F–C.

When results for individual FAA were combined into total (TAA), essential (EAA), non-essential (NEAA) and BCAA groupings (Table 6) it was apparent that for animals fed on diet F–C circulating levels of TAA were consistently, although not significantly, lower than those in animals fed on diet F. This difference in TAA concentration was due to the EAA content of plasma, which was significantly lower for these animals in both carotid and

mesenteric blood samples ($P < 0{\cdot}10$), the NEAA concentration being unaffected by dietary treatment. The major component of the change in EAA concentration was due to a reduction in BCAA in blood obtained from animals fed on diet F–C (carotid and mesenteric $P < 0{\cdot}05$).

## DISCUSSION

The objective of the current study was to establish a situation in which the simultaneous measurement of metabolism within the rumen and across the gut wall could be achieved. Although difficult, this model, which included the use of radio-labelled metabolites, gives some scope for investigation of many aspects of digestion coupled to whole-body metabolic processes.

Rates of VFA production within the rumen are dependent on dietary substrates available for fermentation and the species balance within the microbial population of the rumen to which they are presented. The main difference between animals fed on roughage diets compared with those fed on concentrate diets is the higher proportion of propionate and lower proportion of acetate among the VFA produced in animals fed on concentrates. Growing steers fed on diet F–C in the present experiment showed this change in VFA pattern (Table 1), although the increase in propionate production rate observed was not statistically significant (Table 2). This may in part reflect the poor correlation between VFA molar proportions and measured production rates of individual acids (Sutton, 1985), and also the use of continuous feeding, which has been shown to reduce the difference in VFA fermentation patterns seen between forage and forage–concentrate diets compared with the same diets given twice daily (Yost *et al.* 1977). Calculated propionate production rates for the diets, based on 0·62 organic matter digestibility for diet F and 0·78 for diet F–C (Fitch *et al.* 1989), were 2·32 and 3·19 mol/d; 0·79 and 0·97 of measured values respectively for diet F- and diet F–C-fed steers.

The importance of propionate as a precursor for glucose synthesis in ruminant liver is well established. About 0·53 of whole-body glucose was derived from propionate in the present study (Table 2) and this was unaffected by diet. This value is somewhat higher than estimates reported by other workers for steers fed on forage-based diets (0·34–0·40; Gill & Beever, 1982; Armentano & Young, 1983; Veenhuizen *et al.* 1988). The influence of propionate supply on whole-body glucose turnover rate has been investigated by several groups by either dietary manipulation or supply of exogenous propionate (Bauman *et al.* 1971; Van Maanen *et al.* 1978; Veenhuizen *et al.* 1988). Ulyatt *et al.* (1970) and Herbein *et al.* (1978) have suggested that when energy intakes are balanced glucose turnover rate is unaffected by diet. Although there was a slight increase in glucose turnover rate in diet F–C-fed animals ($P < 0{\cdot}10$, Table 2), the effects of diet on glucose kinetics in the present study are complicated by the difference in protein intakes which has been shown to affect glucose turnover (Judson & Leng, 1968; Lindsay & Williams, 1971). A more comprehensive study with fixed protein intakes and varying propionate supply is needed to resolve this dilemma.

Net absorption rates of VFA into the portal vein from the PDV take no account of metabolism within the gut wall of the rumen or the small intestine. The small net utilization of acetate by MDV in diet F-fed animals (Table 5), for example, suggests a requirement for this nutrient by the small intestine and has been observed by other groups (Bergman & Wolff, 1971; Reynolds & Huntington, 1988*b*). The position of the mesenteric catheter is, however, critical to this observation, since production of acetate by large intestinal fermentation and absorption into the caecal vein may mask net utilization by anterior mesenteric tissues if the sampling catheter is sited beyond the caecal vein–mesenteric vein junction (C. J. Seal and D. S. Parker, unpublished results). Stevens & Stettler (1966) and

Table 5. *Free amino acid concentrations (μmol/l) in carotid (C), mesenteric (M) and portal (P), plasma of steers fed on forage and forage–concentrate diets* and difference between diets at each sampling site*

(Mean values for four animals for C and M (3 df) and three animals for P (2 df))

| Amino acid | | Forage | Forage–concentrate | Difference between diets† | *t* | Statistical significance of difference: $P <$ |
|---|---|---|---|---|---|---|
| Asp | C | 5 | 6 | 0·2 | 0·17 | NS |
| | M | 11 | 10 | −1·2 | 0·58 | NS |
| | P | 9 | 8 | −1·4 | 0·89 | NS |
| Glu | C | 112 | 108 | −4·3 | 1·21 | NS |
| | M | 98 | 109 | 10·8 | 1·49 | NS |
| | P | 95 | 120 | 24·7 | 1·21 | NS |
| Ser | C | 47 | 44 | −2·4 | 0·64 | NS |
| | M | 81 | 77 | −4·0 | 0·37 | NS |
| | P | 67 | 65 | −1·8 | 1·47 | NS |
| Asn | C | 29 | 32 | 2·8 | 0·82 | NS |
| | M | 65 | 67 | 2·2 | 0·20 | NS |
| | P | 49 | 54 | 5·7 | 1·52 | NS |
| Gly | C | 214 | 246 | 31·8 | 1·20 | NS |
| | M | 286 | 314 | 28·0 | 0·74 | NS |
| | P | 217 | 287 | 70·2 | 3·28 | 0·10 |
| Gln | C | 149 | 175 | 29·5 | 1·77 | NS |
| | M | 145 | 198 | 53·1 | 3·40 | 0·05 |
| | P | 91 | 192 | 100·9 | 2·89 | NS |
| Tau | C | 37 | 32 | −4·9 | 1·04 | NS |
| | M | 37 | 39 | 2·1 | 0·41 | NS |
| | P | 33 | 41 | 7·9 | 2·71 | NS |
| His | C | 43 | 45 | 1·9 | 0·33 | NS |
| | M | 52 | 57 | 4·7 | 0·82 | NS |
| | P | 45 | 56 | 10·3 | 0·93 | NS |
| Cit | C | 58 | 55 | −2·5 | 0·23 | NS |
| | M | 61 | 67 | 5·5 | 0·53 | NS |
| | P | 48 | 46 | −1·6 | 0·06 | NS |
| Thr | C | 60 | 55 | −5·6 | 0·62 | NS |
| | M | 97 | 88 | −9·1 | 0·64 | NS |
| | P | 78 | 74 | −3·8 | 0·91 | NS |
| Ala | C | 173 | 182 | 8·6 | 1·02 | NS |
| | M | 299 | 307 | 7·3 | 0·20 | NS |
| | P | 233 | 271 | 38·6 | 1·12 | NS |
| Arg | C | 146 | 89 | −57·0 | 1·46 | NS |
| | M | 178 | 124 | −54·9 | 1·33 | NS |
| | P | 163 | 147 | −15·8 | 0·87 | NS |
| Pro | C | 63 | 80 | 16·9 | 1·96 | NS |
| | M | 87 | 109 | 21·8 | 1·42 | NS |
| | P | 73 | 88 | 14·8 | 3·14 | 0·10 |
| Tyr | C | 23 | 14 | −8·9 | 1·76 | NS |
| | M | 30 | 22 | −6·3 | 0·80 | NS |
| | P | 19 | 23 | 3·8 | 0·56 | NS |
| Val | C | 508 | 298 | −210·0 | 3·78 | 0·05 |
| | M | 524 | 352 | −172·3 | 5·08 | 0·05 |
| | P | 500 | 353 | −146·9 | 2·39 | NS |
| Met | C | 18 | 13 | −4·9 | 4·20 | 0·05 |
| | M | 23 | 29 | 5·7 | 1·21 | NS |
| | P | 24 | 24 | −0·1 | 0·03 | NS |

Table 5. (*cont.*)

| Amino acid | | Forage | Forage–concentrate | Difference between diets† | *t* | Statistical significance of difference: $P <$ |
|---|---|---|---|---|---|---|
| Ile | C | 193 | 121 | −72·0 | 3·57 | 0·05 |
| | M | 234 | 164 | −69·7 | 3·34 | 0·05 |
| | P | 210 | 157 | −53·4 | 2·42 | NS |
| Leu | C | 275 | 204 | −70·2 | 2·72 | 0·10 |
| | M | 317 | 272 | −44·4 | 4·56 | 0·05 |
| | P | 307 | 269 | −38·0 | 0·95 | NS |
| Phe | C | 78 | 69 | −8·7 | 0·70 | NS |
| | M | 162 | 88 | −74·3 | 1·20 | NS |
| | P | 89 | 97 | 8·3 | 0·36 | NS |
| Trp | C | 7 | 5 | −2·1 | 2·55 | 0·10 |
| | M | 30 | 7 | −22·9 | 1·15 | NS |
| | P | 7 | 9 | 1·3 | 0·29 | NS |
| Orn | C | 88 | 71 | −17·6 | 0·97 | NS |
| | M | 91 | 85 | −6·3 | 0·52 | NS |
| | P | 81 | 85 | 4·2 | 0·77 | NS |
| Lys | C | 116 | 81 | −35·5 | 2·49 | 0·10 |
| | M | 166 | 132 | −33·8 | 1·46 | NS |
| | P | 138 | 132 | −6·2 | 0·25 | NS |

NS, not significant (Student's *t* test).
* For details of diet and feeding regimen, see p. 356.
† Forage–concentrate − forage.

Bergman & Wolff (1971) calculated that 0·3-0·45 of the acetate produced in the rumen was metabolized in the rumen wall, and that the proportions of propionate and butyrate metabolized were higher at 0·6 and 0·9, representing a considerable loss of fermentable energy to productive tissues of the animal. Weekes & Webster (1975) showed that between 0·3 and 0·55 of rumen propionate did not appear in the portal vein of sheep and that this was unaffected by different propionate infusion rates into the rumen. The acetate:propionate ratio in rumen fluid for diet F-fed animals was 4·6:1 and fell to 2·9:1 in diet F–C-fed animals. However, the ratio for VFA in portal blood was much higher at 10·8:1 and 7·2:1 for diet F- and diet F–C-fed animals respectively. This may be due to either differences in the efficiencies of absorption of the two VFA across the rumen epithelium or differential metabolism of the VFA by the gut tissue. Propionate arriving at the liver represented only 0·30 of the corresponding rumen propionate production rate for the two diets. Similar calculations based on calculated acetate production rates would suggest that for these animals only 0·48 (diet F) and 0·52 (diet F–C) of acetate production in the rumen could be accounted for by acetate arriving at the liver from PDV. The significant quantity of 3-hydroxybutyrate arising from PDV further emphasizes the importance of the rumen wall in modifying the supply of fermentation end-products to the liver. VFA produced in the rumen and large intestine and glucose absorbed in the small intestine provide additional energy sources which are highly dependent on the extent of fermentation in the rumen and subsequent digestion of carbohydrate along the digestive tract. Net glucose utilization by the MDV and PDV in steers fed on diet F in the present experiment, in contrast to net glucose production in diet F–C-fed animals, is consistent with data from forage-fed ruminants in other studies (Huntington, 1984; Janes *et al.* 1985; Reynolds & Huntington,

Table 6. *Total (TAA), essential (EAA), non-essential (NEAA) and branched-chain (BCAA) amino acid (AA) concentrations, net absorption rates in mesenteric (M)- and portal (P)-drained viscera, and difference between diets for steers fed on forage or forage–concentrate diets**

(Mean values of four animals for C and M AA concentrations (3 df) and three animals for P AA concentrations and net absorption rates (2 df))

| | | Forage | Forage–concentrate | Difference between diets† | *t* | Statistical significance of difference: $P <$ |
|---|---|---|---|---|---|---|
| AA concentration (μmol/l) | | | | | | |
| TAA | C | 2443 | 2024 | −419 | 1·43 | NS |
| | M | 3074 | 2716 | −358 | 1·16 | NS |
| | P | 2577 | 2598 | 22 | 0·07 | NS |
| EAA | C | 1443 | 979 | −464 | 2·62 | 0·10 |
| | M | 1784 | 1313 | −471 | 2·63 | 0·10 |
| | P | 1562 | 1318 | −244 | 1·19 | NS |
| NEAA | C | 1000 | 1045 | 45 | 0·81 | NS |
| | M | 1290 | 1402 | 113 | 0·85 | NS |
| | P | 1014 | 1280 | 265 | 2·13 | NS |
| BCAA | C | 976 | 623 | −352 | 3·48 | 0·05 |
| | M | 1075 | 788 | −286 | 4·76 | 0·05 |
| | P | 1017 | 779 | −238 | 1·94 | NS |
| Net absorption rates (μmol/min) | | | | | | |
| TAA | M | 1033 | 744 | −289 | 1·41 | NS |
| | P | 951 | 1601 | 650 | 0·70 | NS |
| EAA | M | 611 | 383 | −228 | 1·23 | NS |
| | P | 732 | 868 | 136 | 0·26 | NS |
| NEAA | M | 422 | 361 | −61 | 0·63 | NS |
| | P | 218 | 732 | 514 | 1·26 | NS |
| BCAA | M | 89 | 184 | 96 | 0·49 | NS |
| | P | 359 | 415 | 56 | 0·19 | NS |

NS, not significant (Student's *t* test).
* For details of diet and feeding regimen, see p. 356.
† Forage–concentrate − forage.

1988*a*). Net glucose utilization by rumen tissue in diet F-fed animals was 0·16 mmol/min. In animals fed on diet F–C this was reduced to 0·02 mmol/min. Janes *et al.* (1985) and Reynolds & Huntington (1988*a*) also demonstrated net glucose absorption by MDV of concentrate-fed animals and the latter group similarly noted a reduction in total net PDV glucose use in concentrate- rather than forage-fed animals.

Net lactate flux across tissues of the MDV was not affected by dietary treatment (Table 4). In contrast, there was significantly greater lactate production across the PDV for animals fed on diet F compared with diet F–C ($P < 0·05$), Table 4). The proportion of lactate produced by the PDV derived from absorbed lactate or gut wall metabolism of glucose and propionate is not known, although changes in glucose utilization by different regions of the intestine are likely to alter the balance of lactate production from the different substrates available at each tissue site.

Decreased $NH_3$ absorption by MDV and PDV (Table 5) in animals fed on diet F–C may

be attributed to the lower N intake in these animals and the reduction in soluble N sources available in the rumen although, on average for both diets, 0·39 of total PDV $NH_3$ absorption arose from the MDV. Urea extraction from arterial blood by the PDV is a significant proportion of total N removal by the splanchnic bed (Huntington, 1986). Table 4 shows extraction of urea by MDV and PDV in animals fed on both diets, and that this appeared to be greater for diet F-fed compared with diet F–C-fed animals. Although this was not statistically significant, the observation is in agreement with that of Huntington (1988) who demonstrated a similar response in beef steers fed on either lucerne (*Medicago sativa*) hay or a diet containing 800 g cracked maize/kg.

The diets used in the present experiment were designed to be isoenergetic and were fed at a level to give a weight gain of 700 g/d (Agricultural Research Council, 1984). The difference in N content of the two diets did, however, result in a higher N intake for animals when on diet F–C (96 *v.* 57 g N/D). Although in general an increase in N intake has been shown to result in elevated plasma amino acid levels in peripheral blood (Schelling *et al.* 1967; Bergen, 1979), other studies have shown only minor variations in circulating concentrations as a consequence of dietary change (Prior *et al.* 1981). A comparison of the concentrations of individual amino acids in peripheral blood between animals on the two diets in the present study (Table 5) showed that there was a significant reduction in those for valine, methionine and isoleucine ($P < 0·05$) and leucine, tryptophan and lysine ($P < 0·1$) in animals fed on diet F–C, and this was reflected in a significant reduction in the EAA ($P < 0·1$) and BCAA ($P < 0·05$) groups when this diet was given. The decreases in concentration of certain of the BCAA were similar to those reported by Mercer & Miller (1982) following intraruminal infusion of a mixture of VFA in sheep potentially increasing energy availability to peripheral tissues. This is in contrast to the present experiment in which the dietary change resulted in a shift in fermentation pattern in the rumen without any variation in overall VFA concentration (Table 1).

On both diets, concentrations of amino acids in mesenteric and portal blood were consistently higher than in carotid samples, indicating net absorption across the intestinal wall, apart from glutamate, glutamine and taurine which showed a negative V–A difference across the gut in animals fed on diet F. Comparison of net absorption rates determined in the mesenteric and portal veins showed that there was a consistent increase in these values between mesenteric and portal blood in diet F–C-fed animals. Net absorption rates do not provide an absolute measure of metabolite absorption, the value reflecting the overall effect of uptake and metabolism within the tissues. An increase in apparent net uptake of amino acids into portal blood relative to that in mesenteric samples may be a result of lower utilization of amino acids by the mucosal tissues of the forestomachs when compared with that of the small intestine. An alternative to this proposal would be that FAA released as a result of proteolysis of dietary protein and microbial cells within the rumen (Mangan, 1972; Broderick & Wallace, 1988) were absorbed directly across the rumen wall. It is of interest that in the former study accumulation of valine, isoleucine, leucine and lysine was recorded in rumen fluid and these amino acids were quantitatively the major components of the increase in net portal absorption.

Comparison of the net disappearance of amino acids from the gastrointestinal tract with their net appearance in portal blood of sheep (Tagari & Bergman, 1978) has underlined the importance of mucosal tissue metabolism in modifying amino acid availability to the animal. The importance of both glutamate and glutamine in intestinal tissue metabolism is apparent from the negative net portal absorption data reported in sheep (Heitman & Bergman, 1978) and cattle (Huntington & Prior, 1985; Wilton, 1990). These two amino acids have been shown to be the major components of energy utilization by the gastrointestinal mucosa of the rat (Windmueller & Spaeth, 1978, 1980). In the present

experiment net utilization of both glutamine and glutamate by the PDV was apparent in animals fed on diet F. On diet F–C, however, net absorption of glutamine and glutamate occurred and there was a significant difference ($P < 0{\cdot}05$) between the values for glutamine in the mesenteric vein for the two diets, underlining the shift in tissue metabolism of these two amino acids as a result of dietary change. A similar change in glutamine utilization by the PDV in steers transferred from a forage to a concentrate diet (Reynolds & Huntington, 1988*a*) provides further evidence that tissue metabolite utilization alters in these contrasting dietary situations. Net portal appearance of both glutamine and glutamate reported by Prior *et al.* (1981) in their studies with cattle was in contrast to studies with sheep fed on the same diets, where net utilization of the amino acids was recorded, suggesting that in addition to dietary effects there may be species differences in tissue metabolite requirements.

Although only measured over a relatively short period (4 weeks), weight gain by steers on diet F–C was higher than when diet F was given, despite their having a lower N intake. This reduced response on the forage-based diet might be expected from previous studies in which the effects of protein supplementation of forage diets has been studied (see Beever & Siddons, 1985). Inclusion of maize with the dried grass on a 50:50 basis resulted in a shift in rumen fermentation pattern and a reduction in both urea concentration and that of circulating amino acids, when compared with diet F. It is also apparent that manipulation of dietary intake influences intestinal metabolism such that there may be reduced utilization of glutamine and glutamate. The mechanism by which this occurs and its implications for portal amino acid supply merits further study.

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