Effects of different supplements on splanchnic oxygen consumption and net fluxes of nutrients in sheep consuming bromegrass (*Bromus inermis*) hay *ad libitum*

BY A. L. GOETSCH*

Department of Animal Sciences, University of Arkansas, Fayetteville, Arkansas 72701, USA

AND C. L. FERRELL AND H. C. FREETLY

Roman L. Hruska Meat Animal Research Center, Agricultural Research Service, USDA, Clay Center, Nebraska 68933, USA

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Fifteen sheep (53 kg), with catheters in a hepatic vein, the portal vein and a mesenteric vein and artery, were offered a bromegrass (*Bromus inermis*) hay (104 g crude protein (CP), 700 g neutral-detergent fibre and 65 g acid-detergent lignin/kg dry matter (DM)) *ad lib.* with different supplements to determine the effects on net flux of oxygen and nutrients across the portal-drained viscera (PDV) and liver. The sheep were unsupplemented (Control) or received 5 g DM/kg body weight (BW) of ground maize (M), 7 g DM/kg BW of soya-bean hulls (H) or 0·73 g DM/kg BW of a mix of feedstuffs high in rumen-undegradable protein (P). Apparent digestible energy (DE) intakes were 5·3, 10·4, 10·6 and 6·7 (SE 0·74) MJ/d and apparent digestible CP intakes were 37, 50, 79 and 68 (SE 4·3) g/d for Control, M, H and P treatments respectively. Splanchnic tissue oxygen consumption rates were 0·23, 0·32, 0·30 and 0·27 (SE 0·054) mol/h, and oxidative metabolism accounted for 0·46, 0·31, 0·33 and 0·47 (SE 0·051) of DE intakes for Control, M, H and P treatments respectively. Supplements increased ($P < 0·05$) release of α-amino nitrogen (AAN) by the PDV (4·2, 17·5, 19·6 and 18·1 mmol/h for Control, M, H and P treatments respectively). Splanchnic net flux of AAN was not affected by supplement treatments. Hepatic release of urea-N was increased ($P < 0·05$) by supplement treatments (27, 40, 46 and 44 mmol/h for Control, M, H and P respectively); the P treatment increased ($P < 0·05$) and the H treatment tended ($P = 0·10$) to increase splanchnic release of urea-N (7, 10, 20 and 27 mmol/h for Control, M, H and P treatments respectively). Net flux of glucose across the PDV was −4·6, 1·4, −5·6 and −7·2 (SE 1·65) mmol/h for Control, M, H and P treatments respectively. Hepatic glucose released averaged 23 (SE 2·0) mmol/h and was not affected by treatment. Treatments M and H increased ($P < 0·05$) PDV release of propionate compared with the Control treatment (4·5, 15·5, 16·8 and 7·7 mmol/h for Control, M, H and P treatments respectively). Release of acetate by the PDV was 43, 97, 118 and 67 (SE 23·9) mmol/h for Control, M, H and P treatments respectively. In summary, different supplements of low-quality grass did not increase the efficiency of N metabolism by splanchnic tissues. Treatment P had little effect on net flux across splanchnic tissues of glucose, L-lactate, β-hydroxybutyrate and volatile fatty acids (VFA). Overall, treatments M and H had similar effects on splanchnic net fluxes of VFA and L-lactate whereas butyrate and β-hydroxybutyrate releases by the PDV were increased by treatment M.

***Bromus inermis*: Splanchnic metabolism: Forage: Dietary supplement**

Digestible energy (DE) intake and the array of metabolites available to splanchnic tissues affect energy consumption by splanchnic tissues and nutrients available for metabolism by extra-splanchnic tissues (Ferrell *et al.* 1986; Johnson *et al.* 1990; Demigné *et al.* 1991; van der Walt, 1993). A wide variety of feedstuffs is available to supplement forages. Feedstuffs

* For reprints.

high in starch or degradable fibre both increase total digestible energy intakes to extents determined by changes in feed intake and digestibility. High-starch supplements generally increase energy intake, propionate production and intestinal glucose absorption whereas feedstuffs high in rumen-degradable fibre increase energy intake without markedly altering rumen volatile fatty acid (VFA) ratios (Highfill *et al.* 1987; Grigsby *et al.* 1993). Intestinal amino acid absorption is increased by energy supplements and rumen-undegradable protein sources. However, rumen-undegradable protein sources do not markedly affect forage intake, energy absorption or VFA proportions with forages moderate to high in N concentration (McCollum & Horn, 1990). Effects of supplements on portal-drained viscera (PDV) ammonia release and urea uptake and on hepatic urea release and consumption of amino acids are likely to depend largely on relationships between ruminal degradation of nitrogenous compounds and carbohydrates, and may affect energy consumption and efficiency of metabolism by splanchnic tissues. The objectives of this study were to compare effects of supplemental feedstuffs high in starch, rumen-degradable fibre or rumen-undegradable protein on net fluxes of oxygen and nutrients across the PDV and liver of sheep consuming bromegrass (*Bromus inermis*) hay *ad lib.*

MATERIALS AND METHODS

Fifteen Romanov × Composite III (Meat Animal Research Center; 1/4 Suffolk, 1/4 Hampshire and 1.2 Columbia) castrated male sheep (approximately 11 months old) were surgically fitted with chronic indwelling catheters in a hepatic vein, the portal vein and a mesenteric vein and artery (Ferrell *et al.* 1992). Catheter tip lengths were 64 mm for hepatic and portal veins and 380 mm for mesenteric catheters. Catheters were filled with a heparinized saline (9 g NaCl/l) solution (100 U/ml) at surgery and between sampling periods. Some catheters were non-patent at sampling and erroneous portal blood flows occurred for some lambs. Post-mortem examination revealed that such flows resulted from the mesenteric vein catheter residing near the entry point of the portal catheter, presumably resulting in incomplete marker mixing at the tip of the portal catheter. Thus, complete data sets were obtained for seven sheep (one from the control group and two from each of the supplement-treatment groups). The sheep began the experiment 16 to 20 d after surgery (53 (SE 1·1) kg body weight (BW)), being individually maintained in 1·2 × 1·2 m slatted floor pens with free access to water.

Sheep were randomly allocated to four treatments: no supplementation (Control); 5 g DM/kg BW of ground maize (M); 7 g DM/kg BW of soya-bean hulls (H); or 0·73 g DM/kg BW of a mix of feedstuffs high in rumen-undegradable protein (P; see Table 1). Four wethers were subjected to treatments Control, M and H, and three wethers received the P treatment. M and H supplements were fed to provide similar quantities of DE (National Research Council, 1984). The P supplement consisted of 466 g maize-gluten meal, 267 g feather meal and 267 g blood meal/kg DM, providing an amount of supplemental crude protein (CP) intermediate to that of M and H treatments. All sheep were offered coarsely chopped hay, predominantly of bromegrass (no flowering heads), *ad lib.* (120% of previous day's consumption; see Table 1). Supplements were given at 07.30 and 15.30 hours on days 1 to 16 and at 07.30 and 19.30 hours on days 17 to 28 in two equal meals. Supplements were consumed in less than 15 min; hay was offered after supplement ingestion. At each meal, 5 g of a NaCl–trace mineral mixture (988:12, w/w) containing (mg/g): calcium 148, zinc 120, manganese 80, iron 10, iodine 2 and cobalt 1, was sprinkled on the hay.

Forage was consumed *ad lib.* and set quantities of supplement were given in two meals so that conditions would resemble those of common production systems. Similar forage is

Table 1. *Composition of feeds (g/kg dry matter (DM))*

Item	Bromegrass (*Bromus inermis*)	Ground maize	Soya-bean hulls	Protein meals
Ash	116	16	60	18
Crude protein	104	81	167	808
Gross energy (MJ/kg DM)	18·1	18·6	18·7	24·3
Neutral-detergent fibre	695	125	554	265
Acid-detergent fibre	452	21	395	107
Acid-detergent lignin	65	8	21	72
Cellulose	345	13	382	52
Hemicellulose	243	104	159	158

usually consumed *ad lib.* as pasture herbage or as hay offered to ruminants on dormant pasture, and set quantities rather than constant dietary proportions of supplements typically are given. Levels of supplementation in our study were moderate, sufficient for a moderate rate of BW gain by growing animals and for maintenance or a slight increase in BW of gestating ruminants.

Composite feed samples were constructed by daily sampling (days 18 to 28). Faeces were collected in canvas bags on days 21 to 27 (4 d/sheep), with collections starting daily (days 21 to 24) for three or four sheep. Composites were frozen after sampling (100 g/kg). Refusals were sampled (100 g/kg) and composites were formed over a 7 d period (3 d before and during faecal collection). On the last 4 d of the period and on the morning of faecal bag removal, sheep were placed in metabolism cages, with feed and water availability maintained. A priming dose (15 ml) of *p*-amino hippuric acid (PAH; 0·15 M) was given at 2·5 h post-feeding, followed by a constant infusion (0·8 ml/min) of PAH into the mesenteric vein catheter for 6·5 h (2·5 to 9 h post-feeding). BW was determined immediately after sampling.

Blood samples (1 and 10 ml) were withdrawn from portal and hepatic venous and mesenteric arterial catheters hourly from 3 to 9 h post-feeding into heparinized syringes, and placed on ice. Oxygen saturation and haemoglobin concentration were determined immediately with the 1 ml sample using a Hemoximeter (OSM 2; Radiometer Corporation, Copenhagen, Denmark); oxygen concentration was calculated as described by Eisemann & Nienaber (1990). Blood from a 1 ml sample was analysed for glucose with a membrane-immobilized enzyme system involving glucose oxidase (*EC* 1.1.3.4; Model 27, Yellow Springs Instrument Co., Yellow Springs, OH, USA). On the day of sampling, a 1 ml portion of the 10 ml sample was diluted with deionized water (3 ml) and subjected to automated procedures (Technicon Industrial Systems, Tarrytown, NY, USA) for PAH, NH_3-N, urea-N and α-amino nitrogen (AAN), as described by Eisemann & Nienaber (1990). Blood flow for each time was calculated as described by Eisemann & Nienaber (1990). The PAH procedure did not include an acid hydrolysis step; thus, PAH concentration may have been slightly underestimated due to the presence of *N*-acetyl forms. However, the potential error appears small because the hepatic arterial:venous blood flow ratio (Table 2) was similar to that in other studies (van der Walt, 1993).

Remaining blood from the 10 ml sample was frozen. Later, these samples were allowed to thaw and were composited by volume within-animal and across time and vessel. After deproteinization with $Ba(OH)_2$ and $ZnSO_4$ (Yen *et al.* 1991), concentrations of VFA (as described by Yen *et al.* 1991), L-lactate (membrane-immobilized enzyme system involving lactate oxidase (*EC* 1.1.3.2; Model 27, Yellow Springs Instrument Co.) and D-β-

Table 2. *Whole-blood flow, and oxygen and α-amino, urea and ammonia nitrogen measures in sheep consuming bromegrass* (Bromus inermis) ad lib. *with different supplements**

(Mean values and pooled standard error for three observations per treatment)

Item	Treatment				SE
	Control	Maize	Soya-bean hulls	Protein meals	
Blood flow (l/h)					
Portal vein	89	120	120	101	25·4
Hepatic vein	109	143	139	118	26·1
Hepatic artery	20	23	19	17	7·0
Oxygen					
Concentration (mmol/l)					
Portal vein	4·24	4·17	4·64	3·98	0·394
Hepatic vein	3·48	3·28	3·84	3·07	0·393
Artery	5·56	5·52	6·03	5·38	0·423
Consumption (mmol/h)					
Portal-drained viscera	119	164	163	141	34·8
Liver	109	156	132	131	22·5
Splanchnic	228	320	295	271	53·5
α-Amino nitrogen					
Concentration (mmol/l)					
Portal vein	4·59	4·33	4·10	4·59	0·350
Hepatic vein	4·45	4·20	3·92	4·38	0·355
Artery	4·51	4·20	3·93	4·41	0·353
Net flux (mmol/h)					
Portal-drained viscera	7·2	17·5	19·6	18·1	2·98
Liver	−13·2	−15·0	−22·1	−21·7	5·60
Splanchnic	−6·0	2·4	−2·5	−3·5	3·22
Urea nitrogen					
Concentration (mmol/l)					
Portal vein	8·34	6·63	9·09	10·93	0·785
Hepatic vein	8·57	6·94	9·46	11·33	0·797
Artery	8·49	6·85	9·32	11·09	0·781
Net flux (mmol/h)					
Portal-drained viscera	−13·5	−30·4	−26·7	−17·3	8·25
Liver	26·7	40·2	46·3	44·1	5·20
Splanchnic	7·3	9·8	19·6	26·9	4·38
Ammonia nitrogen					
Concentration (mmol/l)					
Portal vein	0·404	0·430	0·486	0·454	0·0352
Hepatic vein	0·278	0·274	0·296	0·254	0·0324
Artery	0·278	0·274	0·294	0·266	0·0298
Net flux (mmol/h)					
Portal-drained viscera	10·6	18·8	23·4	19·1	3·73
Liver	−10·5	−19·2	−23·4	−20·4	4·14
Splanchnic	0·2	−0·4	0·0	−1·3	0·77

* For details of diets and procedures, see Table 1 and pp. 703–705.

hydroxybutyrate (BHBA; Williamson & Mellanby, 1974) were determined. Blood flows and constituent concentrations were averaged over time, and net metabolite fluxes were calculated as described by Burrin *et al.* (1991).

Faecal composites were dried at 55°, allowed to air-equilibrate and ground to pass a 1 mm screen. Feed and refusal samples were ground to pass a 1 mm screen. Feed, refusal and faecal samples were analysed for DM, ash, Kjeldahl N, gross energy (Association of

Official Analytical Chemists, 1984), neutral-detergent fibre (without Na_2SO_3 or decalin), acid-detergent fibre (nonsequential) and acid-detergent lignin (Goering & Van Soest, 1970). Amylase (*EC* 3.2.1.1) was used for neutral-detergent fibre analysis of concentrates, cellulose was estimated as loss in weight upon H_2SO_4 treatment and hemicellulose was the difference between neutral- and acid-detergent fibre fractions. Feed intake on the 2 d before and during faecal collections (6 d total) was averaged across days and used to calculate digestibilities. The mean of feed intake on the day before and the day of blood sampling was used to evaluate relationships of feed intake and net fluxes of oxygen and nutrients.

At 28 d after the first period (Period 1), eight sheep (54 (SE 1·4) kg BW) which previously had patent catheters and appeared to yield reasonable portal blood flows were allocated to different treatments (two per treatment) and subjected to the same procedures. Period 2 lasted 26 d, with blood sampling on the last 2 d of the period. Complete data sets were obtained for five sheep (two from the Control group and one from each of the supplement-treatment group).

Data were analysed by the general linear models procedure of Statistical Analysis Systems (1985) with period, treatment and the treatment × period interaction in the model. Single degree of freedom contrasts were used to compare M, H or P with the Control treatment. Only data sets with complete sets of observations were used.

RESULTS

Total DM intakes were 0·66, 1·01, 1·00 and 0·69 (SE 0·046) kg/d, DE intakes were 5·3, 10·4, 10·6 and 6·7 (SE 0·74) MJ/d, and digestible CP intakes were 37, 50, 79 and 68 (SE 4·3) g/d for Control, M, H and P treatments respectively. Blood flows and oxygen concentrations and consumption rates were not altered by supplement treatments (Table 2). All supplements increased ($P < 0·05$) the release of AAN by the splanchnic bed compared with the Control treatment, but hepatic and splanchnic net fluxes were not affected by treatment. Urea-N uptake by the PDV was similar across treatments, but M, H and P treatments increased ($P < 0·05$) urea-N released by the liver. Urea-N released by the PDV was increased by P ($P < 0·05$) and tended ($P = 0·10$) to be increased by H. Supplementation with H increased ($P < 0·05$) NH_3-N release by the PDV and tended ($P = 0·07$) to increase hepatic uptake.

Concentrations of glucose (Table 3) in portal and hepatic venous and arterial blood were higher ($P < 0·05$) in treatments M and H compared with Control and also tended ($P = 0·10$) to be higher in treatment P. Treatment M had a higher ($P < 0·05$) net flux of glucose across the PDV; however, neither hepatic nor splanchnic release was markedly affected by treatment. The PDV was a net utilizer of glucose and the quantity taken up was relatively constant for Control, H and P treatments. Hepatic release of glucose (20 (SE 2·3) mmol/h) resulted in average splanchnic release of 16 (SE 2·7) mmol/h. Both treatments M and H increased ($P < 0·05$) the concentration of propionate in portal venous blood compared with the Control whereas the concentration in hepatic venous blood was increased ($P < 0·05$) by treatment H but not by treatment M. Treatments M and H similarly increased ($P < 0·05$) propionate released by the PDV and splanchnic tissues and taken up by the liver compared with the Control. L-Lactate concentrations were not affected by treatment; hepatic uptake of L-lactate tended ($P = 0·10$) to be lower for treatment H than for the Control.

Concentrations of acetate in portal and hepatic venous blood were higher in treatment H than in the Control ($P < 0·05$; Table 4). PDV release of acetate tended ($P = 0·07$) to be higher in treatment H. Concentrations of butyrate in portal and hepatic venous blood were elevated ($P < 0·05$) by treatments M and H. Compared with the Control treatment, treatment M enhanced ($P < 0·05$) PDV release and hepatic uptake of butyrate and tended ($P = 0·10$) to increase splanchnic release. Treatment H tended to increase PDV release of

Table 3. *Whole-blood glucose, propionate and L-lactate measures in sheep consuming bromegrass* (Bromus inermis) ad lib. *with different supplements**

(Mean values and pooled standard errors for three observations per treatment)

Item	Treatment				SE
	Control	Maize	Soya-bean hulls	Protein meals	
Glucose					
Concentration (mmol/l)					
Portal vein	2·72	3·11	3·05	2·92	0·073
Hepatic vein	2·89	3·26	3·20	3·10	0·077
Artery	2·77	3·08	3·11	2·99	0·080
Net flux (mmol/h)					
Portal-drained viscera	−4·6	1·4	−5·6	−7·2	1·65
Liver	18·3	23·5	17·2	19·4	5·12
Splanchnic	13·7	24·8	11·7	12·2	4·98
Propionate					
Concentration (mmol/l)					
Portal vein	0·082	0·175	0·180	0·090	0·0215
Hepatic vein	0·028	0·046	0·061	0·026	0·0079
Artery	0·026	0·029	0·032	0·015	0·0053
Net flux (mmol/h)					
Portal-drained viscera	4·5	15·5	16·5	7·7	1·14
Liver	−4·5	−13·0	−13·1	−6·4	1·15
Splanchnic	0·0	2·5	3·6	1·3	0·64
L-Lactate					
Concentration (mmol/l)					
Portal vein	1·45	1·51	1·58	1·48	0·103
Hepatic vein	1·12	1·31	1·41	1·21	0·119
Artery	1·26	1·38	1·48	1·36	0·107
Net flux (mmol/h)					
Portal-drained viscera	18·5	15·6	10·9	11·3	3·58
Liver	−34·0	−24·4	−13·1	−27·7	7·48
Splanchnic	−15·4	−8·9	−2·2	−16·4	6·38

* For details of diets and procedures, see Table 1 and pp. 703–705.

butyrate and to decrease ($P = 0·10$) hepatic uptake. Treatment M tended ($P = 0·06$) to increase BHBA release, although splanchnic release of BHBA was not affected by treatment.

DISCUSSION

Samples were taken 3 to 9 h post-feeding so that periods following meals with elevated blood flow and thereafter when flow declined and stabilized at pre-feeding levels would be represented (Mineo *et al.* 1991). It is probable that effects of time post-feeding on blood flow and nutrient fluxes were similar for treatments M and H. In mature beef cattle consuming grass hay alone or supplemented with M at 210 g/kg diet or H at 260 g/kg diet, neither effects of time post-feeding nor interactions between time and supplement treatment were observed in rumen pH (Galloway *et al.* 1993). Chase & Hibberd (1987) and Martin & Hibberd (1990) supplemented low-quality forages once daily with different levels of M or H and observed patterns of depression in rumen pH with M or H at the same post-feeding sampling times as in this study. Although varying slightly with level of supplementation, pH generally rose at 9 h post-feeding for M and H (Chase & Hibberd, 1987; Martin & Hibberd, 1990). Because of the low rumen-degradability of the P

Table 4. *Whole-blood acetate, butyrate and β-hydroxybutyrate measures in sheep consuming bromegrass* (Bromus inermis) ad lib. *with different supplements**

(Mean values and pooled standard errors for three observations per treatment)

Item	Treatment				SE
	Control	Maize	Soya-bean hulls	Protein meals	
Acetate					
Concentration (mmol/l)					
Portal vein	2·58	3·10	3·47	2·65	0·254
Hepatic vein	2·57	2·91	3·51	2·32	0·254
Artery	2·07	2·16	2·28	1·98	0·287
Consumption (mmol/h)					
Portal-drained viscera	43·4	97·3	118·4	67·3	23·92
Liver	6·9	16·1	18·1	−27·9	21·72
Splanchnic	50·3	113·4	136·6	39·4	41·62
Butyrate					
Concentration (mmol/l)					
Portal vein	0·004	0·026	0·021	0·009	0·0036
Hepatic vein	0·003	0·013	0·012	0·004	0·0027
Artery	0·001	0·006	0·005	0·002	0·0005
Net flux (mmol/h)					
Portal-drained viscera	0·16	2·67	1·83	0·72	0·610
Liver	0·00	−1·56	−1·05	−0·43	0·385
Splanchnic	0·16	1·11	0·78	0·29	0·345
β-Hydroxybutyrate					
Concentration (mmol/l)					
Portal vein	0·60	0·65	0·68	0·56	0·053
Hepatic vein	0·74	0·70	0·72	0·67	0·059
Artery	0·59	0·55	0·61	0·52	0·050
Net flux (mmol/h)					
Portal-drained viscera	2·0	11·8	3·2	3·5	3·03
Liver	15·4	8·6	14·3	14·1	3·31
Splanchnic	17·4	20·4	17·5	17·6	3·36

* For details of diets and procedures, see Table 1 and pp. 703–705.

supplement and its probable pattern of rumen outflow, the pattern of change with time in portal and hepatic blood flows should have been similar for P and Control treatments in this study. Furthermore, the moderate to low levels of supplementation, relatively low forage digestibility and *ad lib.* consumption of forage should have minimized change in blood flow and absorption with time post-feeding compared with higher levels of supplementation and consumption of set, restricted quantities of forage in discrete meals. Thus, the feeding and sampling regimens chosen should be adequate to describe 24 h blood flow and splanchnic net flux of nutrients for the purposes of this study. Additional support for our feeding and sampling regimens is provided by an experiment with lactating dairy cows consuming maize silage–concentrate (60:40) diets *ad lib.* with two meals daily (Reynolds *et al.* 1988). Net fluxes of acetate and propionate across the PDV, liver and total splanchnic tissues determined hourly for 12 h suggest that net flux calculated from samples taken at times used in our study would yield results similar to the average of measures at all times.

Blood flow

Typically, portal and hepatic venous blood flows increase with increasing feed intake (Huntington & Reynolds, 1987). However, blood flow in the period measured was not significantly affected by treatments M or H despite increases in daily DM and DE intakes, although the correlation between DM intake and portal blood flow approached significance (r 0·47; $P = 0·12$). Experiments in which relationships between splanchnic tissue blood flow and level of feed intake have been established generally have entailed relatively large differences in feed intake, less than *ad lib.* consumption and diets higher in digestibility than in our study (Johnson *et al.* 1990). High variability in blood flow and net flux of nutrients across the PDV and liver in our study suggest that with *ad lib.* consumption of a low-quality forage, a high number of observations may be necessary to determine significant differences between supplement treatments compared with restricted feeding of more digestible diets.

Oxygen consumption

Assuming 0·46 MJ of heat energy used per mol of oxygen consumed (McLean, 1972), energy consumption by splanchnic tissues was 0·48, 0·34, 0·30 and 0·46 (SE 0·070) of DE intake for Control, M, H and P treatments respectively. Splanchnic tissues provide a number of service functions for other tissues regardless of level of energy intake. With low energy intakes much of the energy consumed by splanchnic tissues is derived from endogenous substrates, such as free fatty acids and amino acids, rather than from absorbed nutrients. Although the effects of energy supplements on energy consumption were not significant, the effects on the array of metabolites, such as propionate, released by the PDV suggest that they may have led to an increased efficiency of hepatic metabolism.

α-Amino nitrogen

Release of AAN by the PDV in the Control treatment did not differ from zero whilst supplement treatments resulted in marked increases. These findings probably reflect the moderate to low level of protein in the forage, extensive ruminal degradation of the forage protein and limited microbial protein synthesis without energy supplementation. Thus, differences between forage and supplement in level of rumen-undegradable protein and rumen-fermentable organic matter may have elicited change with supplementation, with the former being most important for P and the latter for M and H. Release of AAN by the PDV was 0·25, 0·42, 0·34 and 0·39 (SE 0·063) of N intake for control, M, H and P respectively. The increased release of AAN by the PDV as a proportion of N intake with supplement treatments suggests an increased proportion of ingested N captured in AAN released by the PDV.

These results suggest that feedstuffs high in starch, degradable fibre or rumen-undegradable protein added to a low-quality forage can enhance PDV AAN release to the same degree. Furthermore, because apparently digestible N intake was considerably greater for H than for M, change with energy supplements may be influenced relatively more by characteristics of rumen-fermentable organic matter than by rumen-degradable protein level.

Splanchnic AAN net flux did not differ from zero ($P > 0·10$). Bergman & Pell (1983) concluded that most amino acids released by the PDV are removed by the liver in adult sheep. Extensive hepatic amino acid metabolism relative to PDV release may be due to the relatively high stage of maturity of these sheep and to forage sources low in digestibility and moderate in N concentration. Also, PDV net flux of amino acids as assessed by AAN could have been slightly underestimated because of PDV release of peptides (Webb & Bergman,

1991) and carbon skeletons of amino acids (Lindsay, 1993), and potential binding of free amino acids in blood to larger substances such as albumin.

Urea and ammonia nitrogen

Because the M and H treatments would increase rumen-fermentable organic matter, it was anticipated that PDV NH_3-N release would decline (Huntington & Reynolds, 1987). That this did not occur may relate to a degree of asynchrony in ruminal availability of nitrogenous compounds with that of energy and carbon skeletons liberated in microbial fermentation with the supplement treatments, as well as the moderate concentration of N in the forage.

Hepatic and splanchnic tissue releases of urea-N imply that a considerable proportion of urea-N released by the liver was recycled to the gut in the Control, M and H treatments, with the quantity being lower for treatment H than M. Greater intake of apparently digestible N for treatment H than for treatment M may have been responsible for greater urinary N excretion in treatment H. Urinary excretion may have accounted for a greater proportion of urea-N released by the liver for treatment P than for other treatments. With treatment P, the absence of a marked change in DE intake coupled with an increased apparently digestible N intake presumably yielded a low potential for use of amino acids in peripheral protein synthesis relative to supply, with an ensuing high degree of deamination in the liver.

Overall, splanchnic tissue N metabolism appeared least efficient in treatment P, with elevated NH_3-N release by the PDV, high hepatic urea-N release and low recycling of urea-N to the stomach. Treatments M and H increased hepatic urea-N release and urea-N uptake by the PDV similarly, although with treatment H a slightly lesser proportion of urea-N released by the liver was recycled to the gut because of a higher apparently digestible N intake.

Assuming 4·8 mol of ATP consumed per mol of urea synthesized in the liver (Burrin *et al.* 1991), energy consumed for ureagenesis was 0·11, 0·21, 0·25 and 0·24 (SE 0·028) MJ/d and 0·10, 0·13, 0·17 and 0·17 of total hepatic energy consumption for Control, M, H and P treatments respectively. Substantial increases for all supplement treatments in energy used in ureagenesis, and a similar PDV NH_3-N release:N intake ratio among treatments, suggest potential to decrease liver energy consumption by improving synchrony of fermentation. Another factor which may have contributed to increased energy use in urea synthesis with supplementation was the feeding of a limited quantity of supplement at two discrete times daily. In addition, the low potential for lean tissue accretion of these sheep because of their stage of maturity may have minimized the potential for peripheral tissue protein accretion and demand for amino acids.

Glucose

Escape of starch from the rumen with treatment M presumably was responsible for glucose release by the PDV. Treatment M did not alter hepatic gluconeogenesis. Conversely, Janes *et al.* (1985*a*, *b*) observed that the contribution of endogenous glucose to whole-body turnover was lower in sheep fed on a maize-based diet than in sheep consuming dried grass, although whole-body turnover was greater with the diet high in maize. It was suggested that the concentration of glucose in blood perfusing the liver affected hepatic gluconeogenesis (Janes *et al.* 1985*a*, *b*). Nonetheless, the level of glucose in portal venous blood in our experiment did not correlate with hepatic glucose release.

Supplementation with feedstuffs high in starch, degradable fibre or rumen-undegradable protein did not markedly alter hepatic release of glucose, although elevated PDV net flux

in treatment M tended to increase splanchnic tissue glucose release. Hence, improvements in ruminant performance which might occur under similar conditions in response to supplementation as with treatments H or P are unlikely to involve enhanced peripheral availability of glucose.

Propionate and lactate

Equivalent increases in PDV release of propionate for treatments M and H compared with the Control treatment imply that microbial propionate production was enhanced similarly or that extent of metabolism by ruminal epithelial cells differed. With ruminal propionate infusions, Gross *et al.* (1990) observed constant PDV metabolism of propionate in sheep regardless of infusion level. Conversely, Harmon *et al.* (1993) suggested that propionate oxidation in ruminal epithelium of growing beef steers consuming lucerne (*Medicago sativa*) hay was enhanced to spare arterial glucose from oxidation when monensin increased propionate availability.

Propionate typically is quantitatively the most important substrate for hepatic glucose synthesis (Bergman, 1990), most propionate extracted by the liver is used to form glucose (Weekes, 1991) and the proportion of synthesized glucose arising from propionate normally increases with propionate availability (Baird *et al.* 1980; Elliot, 1980). Weekes (1991) summarized estimates of endogenous glucose arising from propionate which ranged from 0·20 to 0·78. Hepatic glucose release in our study was not altered by treatment regardless of change in PDV release of propionate. The maximal contribution of propionate released by the PDV to hepatic glucose release was 0·12, 0·32, 0·44 and 0·19 (SE 0·056) for Control, M, H and P treatments respectively. Because energy for gluconeogenesis is less with propionate than with other glucose precursors, such as lactate and amino acids (Demigné *et al.* 1991), energy supplements presumably decreased energy used by the liver for gluconeogenesis, with a slightly greater effect in treatment H. The reasons for the relatively low potential glucose synthesis from propionate in this experiment are unclear. Hepatic uptake of L-lactate was high relative to that of propionate, which suggests that a considerable proportion of glucose arose from lactate.

Acetate, butyrate and β-hydroxybutyrate

Though release of acetate by the PDV was not significantly elevated by treatment M or H compared with the Control treatment, DE intake was correlated with acetate release (r 0·61; $P < 0·05$). These results suggest that if energy supplements affect DE intake, PDV release of acetate rises concomitantly.

Increased PDV release of BHBA with treatment M was most likely the result of enhanced microbial butyrate production and ruminal epithelial cell synthesis of BHBA. Butyrate and BHBA releases by the PDV were correlated (r 0·66; $P < 0·05$), presumably because of conversion of butyrate to BHBA in the ruminal epithelium. Increases in PDV release of butyrate with treatments M and H compared with the Control treatment may primarily relate to elevated rumen organic matter fermentation, although greater changes in PDV release of butyrate and BHBA for treatment M than for treatment H could entail a difference in ruminal fermentation pattern relating to an increased number of protozoa (Hall *et al.* 1990; Landis *et al.* 1990).

Energy

The sum of energy from amino acids (based on AAN), L-lactate, BHBA, acetate, propionate, butyrate and glucose released by the PDV (based on heat of combustion values presented by Harmon *et al.* 1991) was 2·16, 4·89, 4·68 and 3·16 (SE 0·600) MJ/d for Control, M, H and P treatments respectively, representing 0·40, 0·46, 0·45 and 0·49 (SE 0·065) of DE

intake. Both treatments M and H ($P < 0.05$) increased the quantity of energy released by the PDV compared with the Control treatment. VFA, primarily acetate, accounted for 0·48, 0·58, 0·67 and 0·56 (SE 0·069) of PDV energy release for Control, M, H and P treatments respectively. Similarly, Harmon *et al.* (1993) observed that acetate, propionate and butyrate accounted for 0·56 and 0·59 of energy released by the PDV of steers fed on lucerne hay without or with monensin respectively.

All supplement treatments decreased energy released by the PDV in L-lactate (0·30, 0·09, 0·08 and 0·12 (SE 0·033) for Control, M, H and P treatments respectively). The P treatment also numerically increased energy released by the PDV in amino acids (based on AAN) compared with the Control treatment (0·17, 0·17, 0·21 and 0·27 (SE 0·037) for Control, M, H and P treatments).

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