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Influence of grass source and legume level on net flux of nutrients across splanchnic tissues in sheep

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Abstract

Crossbred catheterized wethers (20 months old; 45 kg BW, SE 0.7) consumed ad libitum warm ((W) bermudagrass; *Cynodon dactylon*) or cool ((C) ryegrass-wheat mixture; *Lolium multiflorum* and *Triticum aestivum*, respectively) season grass hay with 0%, 20% or 40% legume hay ((L) alfalfa; *Medicago sativa*). Legume, W and C had 54%, 80% and 68% NDF and 17%, 14% and 10% CP, respectively (DM basis). Apparent digestible energy intake was 3.22, 3.42, 3.66, 3.23, 3.57 and 3.67 (SE 0.208) Mcal day^{-1}, and apparent digestible N intake was 17.6, 20.2, 23.4, 10.1, 14.0 and 17.3 (SE 1.09) g day^{-1} for W:0% L, W:20% L, W:40% L, C:0% L, C:20% L and C:40% L, respectively. Splanchnic bed oxygen consumption (306, 364, 380, 261, 316 and 383 mmol h^{-1}; $P = 0.02$) and portal-drained viscera (PDV) releases of alpha-amino N (24.8, 37.6, 38.6, 24.4, 38.4 and 41.7 mmol h^{-1}; $P = 0.04$) and propionate (14.4, 19.4, 20.5, 14.3, 23.6 and 21.1 mmol h^{-1} for W:0% L, W:20% L, W:40% L, C:0% L, C:20% L and C:40% L, respectively; $P = 0.08$) were greater with L than without. However, splanchnic bed net releases of alpha-amino N and glucose were similar among treatments. In conclusion, dietary L inclusion had similar effects on splanchnic tissue oxygen consumption and net fluxes of nutrients regardless of grass source, which differed in concentrations of NDF and CP but not in apparent digestible energy intake. These results suggest that potential improvement of animal performance by substituting 20% or 40% legume for warm or cool season grass, consumed ad libitum by mature ruminants, would not be attributable to change in extra-splanchnic tissue availabilities of energy (based on the difference between apparent digestible energy intake and splanchnic tissue energy consumption), amino acids or glucose.

Keywords: Sheep; Grass; Legume; Metabolism; Net flux

1. Introduction

Warm and cool season grasses provide most nutrients for ruminants throughout the world. Because level of animal productivity may be at times less than desired with grass diets, legumes are often included. However, factors responsible for change in level or efficiency of production with legume inclusion in grass diets are unclear, as well as are effects of grass source and dietary level of legume.

Warm and cool season grasses frequently differ in

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feed intake, digestibility and digestion endproducts. At the same relative stage of maturity, cool season grasses are more digestible than warm season grasses, and feed intake typically is similar or lower for warm than for cool season grasses (Minson, 1990). Primarily because of the lower nonstructural carbohydrate level in warm than in cool season grasses, the acetate:propionate ratio in ruminal fluid is greater for warm season grasses (Minson, 1990). Extent of ruminal degradation of protein in warm and cool season grasses does not appear to differ greatly (Jones et al., 1987; Jones et al., 1988; Brake et al., 1989; Sun et al., 1993), although intestinal amino acid absorption and ruminal volatile fatty acid production may be lower for warm season grasses because of less fermentable organic matter and consequently lower microbial protein synthesis (Hart and Leibholz, 1985; Doyle, 1987).

Intake and organic matter digestibility typically are greater for legumes than for grass diets, and intake of ruminally degradable protein is greater for legumes (Minson, 1990; Waldo et al., 1990; Cruickshank et al., 1992; Galyean and Goetsch, 1993). Efficiency of metabolizable energy use by growing ruminants with intake less than ad libitum is greater for legume than for grass diets (Huntington et al., 1988; Varga et al., 1990; Waldo et al., 1990; Tyrrell et al., 1992), although ad libitum intake of forage-based diets may result in constant efficiency of whole-body energy metabolism regardless of diet quality, with variation rather being expressed in level of intake (Tolkamp and Ketelaars, 1994). Possible factors involved in differences in efficiency of metabolizable energy utilization or feed intake between grass and legume diets include less energy expended in ingestion and rumination of legumes (Minson, 1990), effects of greater propionate absorption with legume diets or associated greater concentrations of propionate in portal blood and (or) glucose in peripheral blood on hormone levels influencing the extent of peripheral tissue protein turnover occurring to support gluconeogenesis (Abdul-Razzaq and Bickerstaffe, 1989), and greater amino acid absorption for legumes that increases peripheral protein accretion (Orskov et al., 1973). The relative importance of such factors should vary with animal characteristics, such as stage of maturity (e.g. mature vs. growing sheep), and magnitudes of difference between grass and mixed grass-legume diets have not been extensively studied.

Because of the high metabolic activity of splanchnic tissues, differences among forages in net flux of nutrients across splanchnic tissues may not necessarily coincide with expectations based on feed intake, digestibility and concentrations of digestion endproducts in the gastrointestinal tract, if factors other than the quantity of absorbed energy affect splanchnic tissue energy consumption or the array of nutrients metabolized. For example, splanchnic tissue energy consumption relative to apparent digestible energy intake (Patil et al., 1995a; Patil et al., 1995b) and gastrointestinal tract mass relative to apparent digestible organic matter intake (Kouakou et al., 1995a; Kouakou et al., 1995b) were greater in growing sheep consuming tropical vs. temperate grass diets, with lower apparent digestible energy intake for tropical grasses. Objectives of this study were to determine influences of level of legume (0%, 20% and 40%) in warm or cool season grass diets consumed ad libitum by mature sheep on feed intake, digestibility and oxygen consumption by and net flux of nutrients across the portal-drained viscera and liver.

2. Materials and methods

Twelve crossbred (Suffolk × Rambouillet-Dorset) wethers (approximately 20 months of age; 45 kg BW, SE 0.7) were surgically fitted with chronic indwelling catheters in a hepatic vein, the portal vein and a mesenteric vein and artery (Ferrell et al., 1992). Catheters were filled with a heparinized (100 U mL^{-1}) saline (0.85%, wt/vol) solution at surgery and between sampling periods. Sheep began the experiment approximately 12 weeks after surgery, being previously used in a 9-week study (44 kg BW, SE 0.7) with warm or cool season grass hay differing in stage of maturity or quality consumed ad libitum (Patil et al., 1995c). Sheep were allotted to treatments randomly. Sheep were individually maintained in 1.2 × 1.2 m slatted floor pens with free access to water and were cared for in accordance with guidelines of Consortium (1988).

Sheep were randomly allotted to four groups with three per group for use in replicated simultaneous

3 × 3 Latin squares, with a split-plot design. Two groups consumed warm season grass (W) and two groups consumed cool season grass (C), with legume (L) hay at 0%, 20% or 40% of the diet. Warm season grass hay was bermudagrass (*Cynodon dactylon*; vegetative growth stage), C hay was ryegrass (*Lolium multiflorum*; late-vegetative to boot stage)-wheat (*Triticum aestivum*; post-anthesis to early milk stage), and L hay was alfalfa (*Medicago sativa*; full-bloom to early seed pod stage). Hay was coarsely chopped at different times throughout the experiment. Sheep consumed hay ad libitum (offered at 105 to 110% of consumption on the preceding few days), with meals at 08:00 h and 16:00 h on Days 1 to 12 and at 08:00 h and 20:00 h on Days 13 to 21. Grass and L hays were thoroughly hand-mixed before each meal. In addition, at 08:00 h sheep received 3.5 g of a mineral mixture of 20% trace minerals (> 12% Zn, 10% Mn, 5% K, 2.5% Mg, 1.5% Cu, 0.3% I, 0.1% Co and 0.02% Se; mineral sources were zinc oxide, ferrous sulfate, manganous oxide, potassium chloride, magnesium oxide, copper oxide, ethylenediamine dihydriodide, cobalt carbonate and sodium selenite) and 80% NaCl.

Composite feed samples were formed by sampling on Days 14 to 21. Feces was collected in canvas bags on Day 15 to 20 (4 days per sheep). Composite samples were formed by subsampling (10%) each day and were frozen between collections. Ruminal fluid was obtained by stomach tube on Day 15 at 12:00 h, strained through cheesecloth, acidified with H_2SO_4, frozen and later analyzed for VFA (Goetsch and Galyean, 1983) and ammonia N (AMN; as described later). On the morning subsequent to removal of fecal bags, sheep (two to four daily) were placed in metabolism crates, with feed and water availability maintained. A priming dose (11.25 mL) of para-aminohippuric acid (4%, wt/vol; pH 7.4) was given at 05:30 h into the mesenteric vein catheter, followed by a continuous infusion (0.6 mL min^{-1}) for 8.5 h. Body weight was determined immediately after the last sample was obtained.

Blood was withdrawn hourly from portal and hepatic venous and mesenteric arterial catheters from −2 to 6 h post-feeding, representing 9 h of the 12 h feed interval. A 1 mL sample was obtained anaerobically into a heparinized syringe and placed in ice. Packed cell volume, oxygen saturation and hemoglobin concentration (OSM 2; Radiometer Corporation, Copenhagen, Denmark) were measured immediately. Oxygen concentration was calculated as described by Eisemann and Nienaber (1990).

A 10 mL sample was taken in a tube with potassium oxalate and sodium fluoride and placed in ice. On the day of sampling, a 1.5 mL aliquot was diluted with deionized water (4.5 mL) and analyzed using automated procedures for para-aminohippuric acid, AMN and alpha-amino N (AAN), as described by Eisemann and Nienaber (1990). Concentrations of para-aminohippuric acid and metabolites were averaged across times, and blood flow was calculated (Eisemann and Nienaber, 1990). Packed cell volume was used to estimate plasma flow. Remaining blood of the 10 mL sample was used to form composites for harvesting of plasma by centrifugation (3.7 *g* for 20 min; stored at −80°C) or for deproteinization with barium hydroxide and zinc sulfate (Yen et al., 1991). Plasma was analyzed for glucose (Sigma Diagnostics, Proc. No. 315; Sigma Chemical Company, St. Louis, MO) and blood deproteinized with barium hydroxide and zinc sulfate for propionate as described by Yen et al. (1991), except that iso-caproic acid was added as an internal standard before gas chromatography. Net metabolite fluxes were calculated based on venoarterial concentration differences and blood flows (Burrin et al., 1991).

Fecal composites were dried at 55°C and allowed to air-equilibrate. Hay and fecal samples were ground to pass a 1 mm screen and analyzed for dry matter, ash, Kjeldahl nitrogen, energy (AOAC, 1984) and NDF (Goering and Van Soest, 1970; without sodium sulfite, decalin or ethoxyethanol). Hay samples also were analyzed for ADF (nonsequential) and ADL (Goering and Van Soest, 1970). Cellulose was estimated as loss in weight upon H_2SO_4 treatment and hemicellulose as the difference between NDF and ADF. The average of feed intake on the 6 days preceding and on the day of blood sampling was used to calculate digestibilities.

Data were analyzed as a split-plot by the General Linear Models procedure of Statistical Analysis Systems Institute Inc. (1990) with a model containing grass source (or square), animal within grass source (error for grass source), period, level of L and the grass source × L level interaction. Orthogonal contrasts were used to test effects of L inclusion, L level

Table 1
Composition (dry matter basis) of forage consumed by sheep

Item	Forage		
	Bermudagrass	Ryegrass-wheat	Alfalfa
Ash (%)	7.2	7.9	8.8
CP (%)	13.9	10.1	16.9
NDF (%)	80.4	67.7	54.1
ADF (%)	33.5	36.3	36.9
ADL (%)	4.7	4.1	8.2
Cellulose (%)	26.6	31.3	28.4
Hemicellulose (%)	46.5	31.3	17.2
GE (kcal kg^{-1})	4482	4379	4343

and interactions between grass source and L inclusion and level. Data from three animals were omitted from analyses because arterial or portal and hepatic venous catheters were non-patent in all periods or were functional only in the first. In addition, for some animals and periods either the portal or hepatic venous catheter was non-patent; there were four observations for which only the portal catheter was patent and four with only a patent hepatic catheter, which consumed C diets. Therefore, the number of observations was 25 (W:0% L, 4; W:20% L, 4; W:40% L, 3; C:0% L, 5; C:20% L, 5; C:40% L, 4) for feed intake, digestion, ruminal fluid concentrations of VFA and AMN and metabolite concentrations in arterial blood. Twenty-one observations were derived for portal blood concentrations and portal-drained viscera (PDV) net fluxes (W:0% L, 4; W:20% L, 4; W:40% L, 3; C:0% L, 4; C:20% L, 3; C:40% L, 3) and for hepatic venous blood concentrations

Table 2
Forage intake and digestion in sheep consuming warm or cool season grass and different levels of legume (L) [a]

Item	Warm season grass			Cool season grass			SE	Effect [b]
	0% L	20% L	40% L	0% L	20% L	40% L		
DM intake (g day^{-1})								
Legume	0	258	570	0	262	544	29.9	I, L
Grass	1127	1047	836	1154	1038	797	52.5	I, L
Total	1127	1305	1406	1154	1300	1342	43.9	I
OM								
Intake (g day^{-1})	1046	1206	1295	1063	1195	1231	40.4	I
Digestion:								
Percent	65.3	61.1	60.6	67.4	65.2	65.7	2.30	
g day^{-1}	684	739	793	708	780	806	44.2	I
GE								
Intake (kcal day^{-1})	5045	5797	6207	5059	5670	5875	194.1	I
Digestion:								
Percent	63.6	58.7	58.1	64.6	63.0	62.5	2.33	i
kcal day^{-1}	3217	3415	3655	3234	3571	3671	207.9	i
NDF								
Intake (g day^{-1})	901	978	970	779	841	838	31.9	I
Digestion:								
Percent	70.1	65.0	61.6	69.2	67.2	64.7	1.93	I
g day^{-1}	632	637	603	534	563	539	32.6	
N								
Intake (g day^{-1})	25.2	30.3	34.6	18.7	24.0	27.4	0.97	g, I, L
Digestion:								
Percent	69.8	66.4	67.2	54.7	58.3	63.3	2.34	G, G*I
g day^{-1}	17.6	20.2	23.4	10.1	14.0	17.3	1.09	G, I, L

[a] The number of observations was 4, 4 and 3 for warm season grass with 0, 20 and 40% L, and 5, 5 and 4 for cool season grass with 0, 20 and 40% L, respectively.[b] Effect: G and g are grass source ($P < 0.05$ and 0.10, respectively); I and i are legume inclusion ($P < 0.05$ and 0.10, respectively); L is legume level ($P < 0.05$); G*I is grass source × legume inclusion interaction ($P < 0.05$).

and splanchnic bed net fluxes (W:0% L, 4; W:20% L, 4; W:40% L, 3; C:0% L, 3; C:20% L, 4; C:40% L, 3). With only six of the 17 observations for hepatic venous blood flow and hepatic net fluxes for C diets, statistical analyses for hepatic arterial blood flow and hepatic net fluxes were not conducted for C diets. Because of non-patent catheters, differences among different blood vessels in concentrations of metabolites may not exactly coincide with net fluxes.

3. Results

3.1. Forage intake and digestion

Crude protein concentration ranked L > W > C, and level of NDF from greatest to least was W, C and L (Table 1). The ratio of ADL:NDF was much greater for L than for W or C (15 vs. 6%).

Grass DM intake was less ($P < 0.01$) with than without L and less ($P < 0.01$) for 40% L than for 20% (Table 2). Total DM intake was increased ($P < 0.01$) by L inclusion but was similar for 20 and 40% L. Decreases in grass intake were 31, 51, 46 and 67% of L intake for W and C with 20 and 40% L, respectively. Dietary L level affected total DM intake similarly, regardless of grass source.

Total tract NDF digestibility was decreased ($P < 0.01$) by L inclusion (Table 2). Apparent digestibility of OM was similar among treatments, and apparent energy digestibility was decreased ($P = 0.10$) by L inclusion. Dietary L inclusion had little effect on total tract N disappearance with W but caused an increase with C (grass source × L inclusion interaction; $P = 0.04$). Apparent digestible OM and energy intakes (DEI) were increased ($P = 0.03$ and 0.04, respectively) similarly by inclusion of L at both levels and were greater for C than for W diets. Digestible N intake was greater for W than for C, increased by L inclusion and greater for 40% L than for 20% ($P = 0.02$), in accordance with differences in forage CP concentration.

3.2. Ruminal fluid parameters

The concentration of AMN in ruminal fluid at 4 h after offering the morning meal was similar among treatments (Table 3). Total VFA concentration in ruminal fluid was greater ($P = 0.03$) for C than for W diets. Grass source also affected ($P < 0.05$) molar percentages of acetate, propionate, isobutyrate, isovalerate and valerate and the acetate:propionate ratio. Molar percentage of acetate was less ($P = 0.04$) and that of propionate greater ($P = 0.06$) with than without L. Overall, the acetate:propionate ratio was decreased by dietary L inclusion, although change was greater for W than for C diets (grass source × L inclusion interaction; $P = 0.05$).

Table 3
Ruminal fluid ammonia N and VFA concentrations in sheep consuming warm or cool season grass and different levels of legume (L) [a]

Item	Warm season grass			Cool season grass			SE	Effect [b]
	0% L	20% L	40% L	0% L	20% L	40% L		
Ammonia N (mg 100 mL^{-1})	15.9	16.8	16.2	18.4	17.8	17.9	1.86	
VFA								
Total (mmol L^{-1})	89	87	85	98	100	101	6.4	G
mol 100 mol^{-1}:								
Acetate	73.9	70.4	69.9	69.2	68.9	66.9	0.85	G, I
Propionate	17.1	19.5	20.2	22.1	21.7	23.4	0.85	G, i
Isobutyrate	0.83	0.90	0.83	0.36	0.48	0.59	0.081	G
Butyrate	6.6	7.4	7.3	7.5	7.8	7.7	0.38	
Isovalerate	0.96	1.01	0.99	0.34	0.47	0.66	0.113	G
Valerate	0.60	0.81	0.86	0.53	0.64	0.76	0.062	G, I
Acetate:propionate	4.29	3.63	3.46	3.17	3.23	2.86	0.167	G, I, g*i

[a] The number of observations was 4, 4 and 3 for warm season grass with 0, 20 and 40% L, and 5, 5 and 4 for cool season grass with 0, 20 and 40% L, respectively. [b] Effect: G is grass source ($P < 0.05$); I and i are legume inclusion ($P < 0.05$ and 0.10, respectively); g*i is grass source × legume inclusion interaction ($P < 0.10$).

Table 4
Whole blood flow, oxygen, alpha-amino N, ammonia N, glucose and propionate values in sheep consuming warm or cool season grass and different levels of legume (L) [a]

Item	Warm season grass			Cool season grass			SE	Effect [b]
	0% L	20% L	40% L	0% L	20% L	40% L		
Blood flow (L h^{-1})								
Portal vein	138	141	133	115	133	126	12.8	
Hepatic vein	164	174	173	150	166	194	19.6	
Hepatic artery	26	34	39				9.5	
Oxygen								
Whole blood concentration (mmol L^{-1}):								
Portal vein	3.53	3.81	3.66	3.83	3.54	3.87	0.267	
Hepatic vein	3.08	3.01	3.00	3.21	3.04	2.87	0.225	
Artery	4.97	5.15	5.22	5.16	4.84	5.05	0.229	
Consumption (mmol h^{-1}):								
Portal-drained viscera	188	191	210	155	155	172	25.3	
Hepatic	117	175	169				14.8	I
Splanchnic	306	364	380	261	316	383	29.3	I
Alpha-amino N								
Whole blood concentration (mmol L^{-1}):								
Portal vein	4.20	4.34	3.98	4.05	4.37	3.65	0.200	L
Hepatic vein	4.07	4.19	3.77	3.83	4.14	3.73	0.257	
Artery	4.02	4.07	3.67	3.87	4.18	3.59	0.191	L
Net flux (mmol h^{-1}):								
Portal-drained viscera	24.8	37.6	38.6	24.4	38.4	41.7	6.91	I
Hepatic	−13.2	−22.7	−24.4				5.83	i
Splanchnic	8.9	16.2	13.4	7.1	6.1	10.7	4.07	
Ammonia N								
Whole blood concentration (mmol L^{-1}):								
Portal vein	0.46	0.43	0.44	0.40	0.40	0.45	0.037	
Hepatic vein	0.28	0.24	0.21	0.25	0.23	0.23	0.037	
Artery	0.31	0.25	0.22	0.28	0.25	0.26	0.031	i
Net flux (mmol h^{-1}):								
Portal-drained viscera	21.0	26.4	29.6	15.2	18.3	20.0	2.73	G, I
Hepatic	−23.5	−28.0	−29.6				0.78	I
Splanchnic	−3.1	−1.0	−0.3	−3.0	−4.0	−3.8	1.02	
Glucose								
Plasma concentration (mmol L^{-1}):								
Portal vein	3.12	3.07	3.17	3.08	3.24	3.05	0.104	
Hepatic vein	3.35	3.34	3.38	3.45	3.44	3.48	0.113	
Artery	3.17	3.17	3.21	3.20	3.23	3.16	0.106	
Net flux (mmol h^{-1}):								
Portal − drained viscera	−4.9	−8.7	−5.1	−7.3	−3.1	−5.8	2.27	
Hepatic	27.4	29.8	26.1				4.01	
Splanchnic	22.9	20.6	21.5	27.1	32.3	31.7	4.51	g
Propionate								
Whole blood concentration (mmol L^{-1}):								
Portal vein	0.110	0.145	0.168	0.136	0.184	0.180	0.0231	I
Hepatic vein	0.020	0.023	0.033	0.034	0.021	0.027	0.0074	
Artery	0.013	0.015	0.016	0.016	0.015	0.016	0.0015	

Table 4 (continued)

Item	Warm season grass			Cool season grass			SE	Effect [b]
	0% L	20% L	40% L	0% L	20% L	40% L		
Net flux (mmol h^{-1}):								
Portal-drained viscera	14.4	19.4	20.5	14.3	23.6	21.1	4.01	i
Hepatic	−12.5	−18.0	−17.6				2.77	
Splanchnic	1.1	1.6	2.8	2.0	1.5	1.8	1.04	

[a] The number of observations for all variables was 4, 4 and 3 for warm season grass with 0, 20 and 40% L. The number of observations for cool season grass was 5, 5 and 4 with 0, 20 and 40% L for arterial concentrations; 4, 3 and 3 with 0, 20 and 40% L for portal concentrations and portal-drained viscera net fluxes; and 3, 4 and 3 for hepatic concentrations and splanchnic net fluxes.[b] Effect: G is grass source ($P < 0.05$); I and i are legume inclusion ($P < 0.05$ and 0.10, respectively); L and l are legume level ($P < 0.05$ and 0.10, respectively).

3.3. Net fluxes

Portal and hepatic venous and hepatic arterial blood flows were similar among treatments (Table 4). Portal-drained viscera oxygen consumption was not affected by dietary treatment; hepatic oxygen consumption for W was greater ($P = 0.05$) with than without L, and splanchnic bed oxygen consumption was similarly increased ($P = 0.02$) by dietary L inclusion with both grass sources.

Net release of AAN by the PDV was increased ($P = 0.04$) similarly by 20% and 40% L (Table 4). Net hepatic uptake and splanchnic net release of AAN for W were not affected by L inclusion or level. Portal-drained viscera net release of AMN was increased ($P = 0.05$) by L inclusion in diets similarly with W and C. Likewise, with W dietary L inclusion increased ($P = 0.01$) hepatic AMN net uptake. Splanchnic bed AMN net flux was similar among treatments.

Glucose concentration in arterial blood and net fluxes across the PDV and liver did not differ among treatments (Table 4). However, splanchnic bed net release of glucose was greater ($P = 0.10$) for C than for W diets. Propionate concentration in portal venous blood concentration ($P = 0.05$) and PDV net release ($P = 0.08$) were increased by L inclusion in diets, and splanchnic bed net release of propionate was not affected by dietary treatments.

4. Discussion

4.1. Intake and digestion

Typically, intake of C by growing ruminants is higher than that of W (Minson, 1990); however, energy and nutrient demands affect feed intake (Jarrige et al., 1986; Gherardi and Black, 1989; Webster, 1993). These wethers, 20 months of age, did not have a high potential for, or expend a great deal of energy in, peripheral protein accretion, which may have contributed to similar intake of W and C diets. In addition, NDF digestibility was similar for W and C diets, implying different relative stages of maturity at harvests.

The greater degree of lignification of cell walls in L than in grasses may account for lower digestibilities of OM, energy and NDF for diets with L. In addition, ruminal digesta retention time typically decreases with increasing feed intake (Owens and Goetsch, 1986) and is shorter for L than for grass diets (Moseley and Jones, 1979; Moseley and Jones, 1984). Thus, dietary L inclusion may have decreased ruminal digesta retention time, which limited digestion extent. However, greater intake of diets with L than without more than compensated for less extensive digestion. Intake of legumes frequently is greater than that of grasses (Goering et al., 1991; Thomson et al., 1991; Cruickshank et al., 1992), with possible responsible factors of more rapid ruminal digestion and digesta outflow (Beever et al., 1986a; Beever et al., 1986b; Moore and Cherney, 1986; Akin, 1989) and differences in the array of absorbed digestion endproducts, assuming physiological regulation of feed intake (Glenn et al., 1989; Waldo et al., 1990; Tolkamp and Ketelaars, 1992).

4.2. Ruminal fluid parameters

Similar ruminal AMN concentration for W and C diets was not anticipated considering the higher CP concentration in W. In accordance, total VFA con-

centration in ruminal fluid was greater for C than for W diets even though DEI was similar. These findings imply that ruminal digesta volume was greater for W than for C diets, as noted by Reid et al. (1990) and Sun et al. (1994). No effect of dietary L inclusion or level on ruminal AMN concentration may reflect greater ruminal microbial protein synthesis with L, resulting from the lower NDF concentration and more rapid digestion of potentially digestible cell walls in L than in grasses (Moseley and Jones, 1979; Beever et al., 1986a; Beever et al., 1986b).

Minson (1990) summarized that differences between W and C in molar percentages of acetate and propionate, as observed in our experiment, relate to greater nonstructural carbohydrate concentration in C. Lower acetate:propionate ratios in ruminal fluid for diets consisting of L vs. grasses have been observed in other experiments, although such differences could relate more to lower levels of cell walls or fiber in L rather than to concentrations of nonstructural carbohydrate per se (Minson, 1990). Accordingly, the greater effect of L inclusion on the acetate:propionate ratio with W than with C diets probably involves the greater concentration of NDF in W. Assuming similar ruminal digesta volume among dietary L levels, that L inclusion did not change total VFA concentration in ruminal fluid despite increased DEI implies increased partitioning of energy to microbial cells rather than to their digestion endproducts, which is supported by greater PDV AAN release for diets with L than without.

4.3. AAN and AMN net fluxes

Greater PDV net release of AAN with than without dietary L corresponds to greater DEI and the postulate that ruminal microbial outflow was enhanced by L inclusion. Change in PDV AAN net release may have been a function of greater ruminal OM fermentation, as greater PDV AMN net release with L inclusion accounted for much of the increase in digestible N intake and legume protein is highly degradable in the rumen (Akin, 1989; Galyean and Goetsch, 1993; Wilson, 1993). Hepatic AAN net uptake was increased by L inclusion in W diets, and the difference between PDV and splanchnic bed net releases of AAN with C diets suggests an effect of L inclusion similar to that with W. That the effect of L inclusion on PDV release of AAN was not associated with increased splanchnic release may relate to low potential for amino acid use in peripheral protein accretion by these wethers. Likewise, with restricted levels of feed intake, greater hepatic AAN net uptake with than without abomasal casein infusion (Guerino et al., 1991) and for a diet of chopped alfalfa hay with greater N intake than for lower N intake with a high-concentrate diet (Huntington, 1989) were observed. However, in addition to the influence of peripheral tissue demand for amino acids to support protein accretion, Guerino et al. (1991) suggested that such responses may be functions of increased use of amino acids in hepatic protein synthesis to accomodate greater nutrient metabolism and energy consumption by the liver.

The difference between grass sources in, and the effect of L inclusion on, PDV net release of AMN were expected based on total and apparent digestible N intakes. Net release of AMN by the PDV was 49%, 51%, 49%, 50%, 47% and 44% (SE 5.3) of nitrogen intake for W:0% L, W:20% L, W:40% L, C:0% L, C:20% L and C:40% L, respectively, suggesting similar ruminal degradability of protein in both grass sources.

4.4. Glucose and propionate net fluxes

It is generally thought that glucose metabolized by the PDV increases with increasing physiological workload (Balcells et al., 1995). However, possibly because of relatively small differences in DEI among diets in our study, dietary L inclusion did not influence PDV net uptake of glucose, and PDV net uptake of glucose and DEI were not correlated.

Similar PDV net release of propionate for W and C diets despite a greater molar percentage of propionate in ruminal fluid for C diets may reflect greater ruminal digesta volume for W diets, as suggested earlier. Greater PDV release of propionate with than without L appeared due primarily to the greater molar percentage of propionate in ruminal fluid. Thus, ruminal epithelial cell metabolism of propionate relative to the quantity available did not seem markedly altered by dietary L inclusion. That increased PDV propionate release with L inclusion was not associated with increased hepatic or splanchnic net releases of glucose may reflect in part a

limited glucose demand in peripheral tissues because of moderate DEI accompanying the absence of dietary concentrate.

4.5. Oxygen consumption

Differences between oxygen consumption by the PDV and splanchnic bed suggest that the effect of L inclusion in C diets on hepatic oxygen consumption was similar to that with W diets. It appeared that dietary L inclusion altered splanchnic bed oxygen consumption primarily via change in liver metabolism. Physiological workload, or the quantity of energy absorbed, is thought to be the primary determinant of heat energy production by the PDV and liver with levels of feed intake less than ad libitum (Johnson et al., 1990). Accordingly, greater hepatic energy consumption was expected for diets with L than without because of greater DEI. However, changes in splanchnic and hepatic (W diets) oxygen consumption with L inclusion were relatively greater than that in DEI.

Factors responsible for the relatively greater increase in splanchnic bed energy consumption than in DEI with dietary L inclusion are unknown. The increase in PDV net release of propionate with dietary L inclusion and no effects of L inclusion on splanchnic or hepatic (W diets) net releases of glucose indicate that energy used in gluconeogenesis was not increased. Greater PDV net releases and hepatic net uptakes (W diets) of AAN and AMN with than without L suggest an increase in the quantity of energy used in ureagenesis, but of a magnitude considerably less than increases in splanchnic bed and hepatic energy consumption. Thus, energy consumed by the liver in other processes such as ketogenesis, protein turnover, phospholipid and cholesterol synthesis, release of lipoproteins, plasma proteins and peptide hormones, synthesis and release of bile, degradation of plasma proteins and peptide hormones, assimilation of bilirubin and detoxifications (Danfaer, 1994) may have contributed to the increase in splanchnic energy consumption with dietary L inclusion.

Splanchnic bed energy consumption (assuming 110 kcal mol^{-1} oxygen; McLean, 1972) represented 25.6%, 29.0%, 29.6%, 21.5%, 23.7% and 27.2% (SE 4.11) of DEI for W:0% L, W:20% L, W:40% L, C:0% L, C:20% L and C:40% L, respectively. The difference between splanchnic tissue energy consumption and DEI, relating directly to energy available to extra-splanchnic tissues, was 2.41, 2.46, 2.65, 2.57, 2.62 and 2.66 (SE 0.353) Mcal day^{-1} for W:0% L, W:20% L, W:40% L, C:0% L, C:20% L and C:40% L, respectively. Therefore, these results do not indicate that performance (e.g., live weight gain or feed efficiency) under similar conditions would be improved by dietary L inclusion, although energy used for mastication during ingestion and rumination might have been affected in our experiment. Alternatively, if L inclusion enhanced performance, the change would not have been elicited by an increase in the quantity of energy becoming available to peripheral tissues. Perhaps with growing ruminants that have greater energy and nutrient demands than animals used in our experiment, or with other experimental conditions affecting energy expended in functions such as activity (e.g., pasture conditions), a benefit of dietary L inclusion to extra-splanchnic tissue energy availability might occur.

Because splanchnic tissue heat production accounts for approximately 50% of that of the whole-body (Ferrell, 1988), it is conceivable that dietary L inclusion altered efficiency of peripheral energy metabolism, which was not evaluated in our study. An example of a potential effect of dietary L inclusion on peripheral tissue heat production relative to energy metabolized, implicated by results in our experiment, involves altered hormonal conditions. Portal blood propionate concentration, which was increased by L substitution for grass, impacts insulin secretion (Kelly et al., 1993). Ketelaars and Tolkamp (1992) suggested that elevated blood insulin increases efficiency of adipocyte metabolism by increasing intracellular glucose concentration, associated with increased NADPH availability for lipogenesis, and by increasing intracellular pH for enhanced lipogenic enzyme activity. These changes increase acetate use in lipogenesis, thereby decreasing heat energy generated in proton expulsion from cells and (or) substrate cycles to dissipate ATP generated from oxidation of excess acetate (Leng et al., 1993). In addition, elevated blood insulin may alter efficiency of energy metabolism by lessening peripheral tissue protein degradation (Abdul-Razzaq and Bickerstaffe, 1989).

5. Conclusion

Effects of L inclusion in diets consumed ad libitum by 20-month-old wethers were similar for the two grass sources, which differed in chemical composition (e.g. concentrations of NDF and CP) though not in DEI. The small to moderate increase in DEI when L was included in diets was compensated for by elevated splanchnic bed energy consumption, regardless of L level. Legume inclusion increased PDV net release of AAN presumably via greater ruminal microbial protein synthesis associated with increased DEI. Net release of AMN by the PDV was greater with than without L most likely because of insufficient ruminally fermentable OM relative to degraded protein. Greater hepatic net uptakes of AAN and AMN with than without L may have contributed to, but not totally accounted for, the increase in splanchnic bed heat energy production. Portal blood concentration and PDV net release of propionate were increased by L addition to diets. However, neither splanchnic bed net release nor arterial plasma concentration of glucose was altered by dietary L inclusion. These results suggest that under these experimental conditions, factors other than energy consumption by splanchnic tissues or the quantity of energy available to peripheral tissues would be responsible for any improvement in level or efficiency of ruminant production resulting from L inclusion in grass diets.

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