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Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep

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1. Six closely shorn sheep were given brome grass (*Bromus inermis*) pellets at the rate of 59 or 98 g dry matter (DM)/h and maintained at ambient temperatures of 2–5° and 22–25° for 35 d. Measurements of digestion, rate of passage of digesta, and nitrogen transformations were made during the last 13 d of temperature exposure.

2. Cold exposure at the lower level of intake reduced the apparent digestibility of DM and organic matter (OM) approximately 0·055 units. Apparent digestibility of DM and OM was further decreased approximately 0·03 units with the higher level of food intake in the cold. Apparent N digestibility was significantly depressed from 0·62 to 0·59–0·60 for sheep exposed to cold at both levels of intake.

3. Exposure of sheep to cold resulted in a decrease in the turnover time of the particulate marker, ^{103}Ru, from 19 h to 10–12 h in the rumen, a decrease in rumen volume, and a significant increase in DM and OM which escaped digestion in the stomach. Volatile fatty acid and methane production in the rumen were highly correlated with the amount of OM digested in the stomach. Methane production in the rumen comprised 0·81 of total production in warm sheep, and 0·68–0·74 of total production in cold-exposed sheep.

4. More OM and non-ammonia-N were apparently digested in the intestines of sheep exposed to cold than in warm sheep at the same food intake, but the apparent digestibilities in the intestines of DM, OM and non-ammonia-N leaving the abomasum did not change significantly between treatments. The retention time of ^{103}Ru in the intestines was 17–18 h in sheep given 59 g DM food/h at both exposure temperatures, but was reduced to 12 h for cold-exposed sheep given 98 g DM/h. Methane production in the postruminal tract was increased at the higher food intake, but there was no difference between warm and cold-exposed sheep at the same food intake.

5. The rate of irreversible loss of plasma urea and rumen ammonia was measured by infusion of [^{15}N]urea and [^{15}N]ammonium chloride. Exposure to cold reduced the irreversible loss of plasma urea from 0·85 to 0·75–0·77 g N/g N intake, and the irreversible loss of rumen ammonia from 0·66 to 0·57–0·61 g N/g N intake. The transfer of plasma urea-N to the rumen ammonia pool was significantly greater (9·5 g N/d) in the cold-exposed sheep than the value (7·3 g N/d) in warm sheep.

6. The efficiency of microbial synthesis in the rumen was increased in cold-exposed sheep, and was related to the amount of N recycled through the rumen ammonia pool from intraruminal sources. The effect of dilution rate and fermentation patterns on efficiency of microbial synthesis is discussed.

Prolonged cold exposure of cattle or closely shorn sheep to the natural winter environment of western Canada or in climate chambers results in a depression of apparent dry matter (DM) digestibility by about 0·002 units per degree (Christopherson, 1976; Kennedy, Christopherson & Milligan, 1976; Westra & Christopherson, 1976) in association with an increased rate of passage of digesta through the reticulo-rumen (Kennedy *et al.* 1976; Westra & Christopherson, 1976). The increased rate of passage resulted in an increase in efficiency of microbial synthesis and an increase in the quantity of food N which escaped digestion in the rumen (Kennedy *et al.* 1976).

The present experiment was designed to provide further information on digestion and N metabolism in sheep at two exposure temperatures given brome grass (*Bromus inermis*) pellets, at two levels of food intake. In addition, estimates of the rates of production of volatile fatty acids (VFA) and methane in the rumen were made. The rates of movement of N through the rumen ammonia and plasma urea pools, and the rate of transfer of plasma urea-N to rumen ammonia were measured by tracer techniques using ^{15}N.

EXPERIMENTAL

Sheep and their management

Six Suffolk wethers, 2 years old, and initially weighing 59–69 kg, were fitted with cannulas in the rumen and abomasum. Sheep were individually housed in metabolism cages with two sheep in each of three climate chambers, with continuous illumination. Two chambers were maintained at 2–5° (cold), and the other at 22–25° (warm). Each sheep had been treated for internal and external parasites, and was trained to accept the experimental manipulations of sampling. At intervals of 18 d, all sheep were closely shorn, weighed, and injected with retinol, cholecalciferol and α-tocopherol. After 35 and 70 d, the sheep were transferred to another treatment according to the experimental design.

Diet and feeding

The brome grass was ground through a 5 mm screen before pelleting, and contained approximately 120 g crude protein (N × 6·25)/kg DM. The ration was given in equal portions at 1 h intervals, using an automatic feeding apparatus. Water was available *ad lib.*, and cobalt-iodized salt (10 g/d) was given between 20.00 and 20.30 hours.

Conduct of experiment

During each of three periods of 35 d, each sheep was sampled according to the following schedule. After a temperature adaptation period of 21 d, on day 22 all sheep were given a single injection of ^{103}Ru-labelled Tris-(1,10-phenanthroline)-ruthenium (II) chloride (^{103}Ru-P; Tan, Weston & Hogan, 1971) into the abomasum in 5 ml (3–4 μCi/ml) and washed in with 10 ml water. Faeces were collected at the following intervals after injection: 3 hourly for 15 h, 6 hourly from 15 to 36 h, 12 hourly from 36 to 72 h. On day 23, three sheep, A, B and C, received an intraruminal injection (100 ml) of [^{15}N]NH_4Cl (250 mg, 99 atoms ^{15}N/100 atoms N) and ^{51}Cr complexed with EDTA (^{51}Cr-EDTA, 150 μCi) (Downes & McDonald, 1964) dissolved in water. The solution was directed into various parts of the rumen in order to effect rapid mixing. Rumen fluid (20 ml) was sampled at intervals for 48 h. The remaining three sheep, D, E and F, received an intravenous infusion through catheters inserted into the jugular vein of [^{15}N]urea (100 mg/d, 96 atoms ^{15}N/100 atoms N, 1·8 mg/ml) in physiological saline (9 g NaCl/l) for 48 h. Samples of blood (10 ml), from a second catheter inserted in the other jugular vein, and rumen fluid (20 ml) were taken at 1 h intervals from 40 to 48 h after the start of the continuous infusion.

On days 25–27, the rates of VFA and methane production in the rumen were measured using sodium [^{14}C]acetate and [^{3}H]methane for each animal over a 3 h period as described by Weller, Gray, Pilgrim & Jones (1967) and Murray, Bryant & Leng (1976) respectively.

On day 28, a priming dose of ^{51}Cr-EDTA (120 μCi) and ^{103}Ru-P (15 μCi) was given into the rumen, followed by a continuous infusion (500 ml/d; ^{51}Cr-EDTA, 120 μCi/d; ^{103}Ru-P, 15 μCi/d; [^{35}S]Na_2SO_4, 60 μCi/d) to allow measurement of flow of digesta and microbial material through the abomasum as described previously (Kennedy *et al.* 1976). Three sheep, D, E and F, also received [^{15}N]NH_4Cl (99 atoms ^{15}N/100 atoms N, 200 mg/d). Samples of blood, rumen fluid and abomasal contents were taken on days 32–34. The infusion was stopped at 08.00 hours on day 35 and the rates of disappearance of ^{51}Cr from rumen fluid, and of ^{103}Ru from abomasal digesta were determined over 10 h.

Faeces and urine were collected for 7 d (days 22–28) for digestibility and retention measurements.

The experimental design consisted of two 3 × 3 Latin squares run concurrently, with two groups of sheep comprising sheep A, B and C, and D, E and F respectively. The treatments

were designated as WL, CL and CH to denote warm-exposed (W) and cold-exposed (C) sheep, and low (L) and high (H) level of feeding respectively.

Sampling and laboratory procedures

Procedures for sampling from the abomasum and rumen have been described previously (Kennedy *et al.* 1976).

Samples of saliva were taken by suction from the top of the oesophagus of all sheep on ten occasions during days 32–34. Samples contaminated with ingesta were discarded.

For measurement of methane production, sheep were fitted with a flatus collection apparatus. Room air was drawn (30 l/min) across the anus and from a funnel fastened around the rumen fistula and passed into the hood which enclosed the head of the sheep. Food pellets were given at 1 h intervals through a funnel sealed into the top of the hood. The flow of air from the hood (approximately 70 l/min) was adjusted to maintain the concentration of CO_2 below 0·01 units. Samples of rumen fluid, obtained by suction through a plastic tube covered by fine nylon gauze, were immediately treated with two drops conc. sulphuric acid (added to inhibit bacterial action and to fix free ammonia), and stored at $-15°$ before analysis for ^{15}N. Plasma was separated by centrifugation of heparinized blood and stored at $-15°$.

Analytical methods

DM was determined by heating at 65° to constant weight. Other methods for determination of organic matter (OM), sulphate, total S, ^{35}S, total N, VFA concentration, individual VFA proportions, ^{51}Cr and ^{103}Ru were described previously (Kennedy *et al.* 1976). Cell wall constituents (CWC) were determined by analysis for neutral detergent fibre (Van Soest & McQueen, 1973).

The specific radioactivity of [^{3}H]methane was determined using a 4-l ion chamber attached to a Cary vibrating-reed electrometer (model 401; Cary Instruments, Monrovia, Calif., USA) and a Beckman non-dispersive infra-red gas analyzer (Beckman Instruments Inc., Fullerton, Calif., USA).

Phosphate in feed, saliva and rumen fluid was estimated by a colorimetric method (Association of Official Analytical Chemists, 1975).

Ammonia in rumen fluid was collected by steam distillation over magnesium oxide and titrated with 0·005 M-H_2SO_4. Urine and deproteinized plasma samples were treated similarly after addition of urease (Nolan & Leng, 1972) in order to isolate urea-N. ^{15}N was measured using a mass spectrometer (model CEC 21-614; Dupont Instrument Products, Newtown, Conn., USA) and N_2 samples prepared from $(NH_4)_2SO_4$ by the method of Francis, Mulligan & Workmall (1959). Background abundance of ^{15}N in samples from each sheep was estimated before infusion of ^{15}N and the precautions outlined by Nolan & Leng (1972) were observed during sample preparation. The concentration of urea in plasma was determined using the urease method of Fawcett & Scott (1960).

Calculations

Movement of digesta and microbial material through the abomasum was calculated with reference to ^{51}Cr-EDTA, ^{103}Ru-P and ^{35}S as described previously (Kennedy *et al.* 1976). The rate of irreversible loss (production rate) of rumen VFA, methane, ammonia, and plasma urea were estimated at 'plateau' using standard procedures (Shipley & Clark, 1972); 'plateau' enrichment of plasma urea and urinary urea did not differ. The proportions of rumen ammonia derived from plasma urea, and of plasma urea from rumen ammonia were calculated from the 'plateau' enrichment ratios, and the transfer of plasma urea-N to rumen ammonia was calculated as described previously for sulphate transfer (Kennedy &

Table 1. *Intake and digestibility in the gastrointestinal tract of dry matter* (DM), *organic matter* (OM), *cell wall constituents* (*CWC*), *and intake, digestibility, and retention of nitrogen by sheep maintained at two temperatures and at two levels of food intake. WL and CL designate sheep maintained at* 22–25° *and* 2–5° *respectively given* 1410 g DM *food/d. CH designates sheep maintained at* 2–5° *and given* 2350 *g* DM *food/d*

(Mean values with their standard errors for six sheep/treatment)

	Treatments			
	WL	CL	CH	SE of mean
Intake (g/d):				
Water	3340	1860	3750	80
DM	1410	1410	2350	3·9
OM	1310	1310	2180	3·5
CWC	904	904	1510	2·5
N	28·1	28·1	46·9	0·18
Urinary N (g/d)	13·9	14·1	23·5	2·02
N retention (g/d)	3·5	2·4	4·0	0·62
Digestibility:				
DM	0·574	0·518	0·484	0·0062
OM	0·581	0·528	0·489	0·0036
CWC	0·559	0·488	0·435	0·0042
N	0·618	0·597	0·586	0·0025
Body-wt (kg)*	59	51	57	1·0

* Body-weight after 35 d on treatment.

Milligan, 1978). The total entry rate and irreversible loss rate of rumen ammonia were calculated after single injection of $[^{15}N]NH_4Cl$ as described by Nolan & Leng (1974).

Rumen fluid volume, dilution rate of ^{51}Cr-EDTA, water turnover in the rumen, and turnover of ^{103}Ru-P in the stomach were calculated assuming first-order kinetics (Shipley & Clark, 1972). Retention time of ^{103}Ru-P in the intestines was calculated using equation (8) of Faichney (1975).

Saliva production was calculated by reference to phosphate as described by Hemsley, Hogan & Weston (1975).

Statistical analysis

The results were analysed by analysis of variance using the Student–Newman–Keuls' test to test for significance of differences between means (Steel & Torrie, 1960).

RESULTS

Digestion in the gastrointestinal tract

Exposure of shorn wethers to cold (CL treatment) resulted in significant ($P < 0.001$) reductions in the apparent digestibility of DM, OM and CWC when compared to maintenance in the warm (WL treatment) at constant food intake (Table 1). Reductions ($P < 0.001$) in DM, OM and CWC apparent digestibility were associated with increased food intake of cold-exposed sheep (CH *v* CL treatments). N retention did not vary significantly between treatments, but urinary N excretion was significantly higher ($P < 0.05$) in the CH treatment than in the CL or WL treatment, and the apparent N digestibility was significantly higher ($P < 0.001$) in warm sheep than in cold-exposed sheep.

Table 2. *Digestion in, and flow from the stomach of dry matter (DM), organic matter (OM), cell wall constituents (CWC) and nitrogen, rate of passage of digesta from the rumen, and efficiency of microbial synthesis in the rumen of sheep given brome grass (Bromus inermis) pellets*

(Mean values with their standard errors for six sheep/treatment)

	Treatments*			
	WL	CL	CH	SE of mean
Flow through abomasum (g/d) of:				
Digesta	15300	16000	24900	1000
DM	896	1080	1810	24·9
OM	762	916	1585	21·7
CWC	441	530	993	18·4
Ammonia-N	1·4	1·1	1·6	0·20
Non-ammonia-N	30·9	34·8	54·3	0·87
Apparent loss in stomach (g/d) of:				
DM	514	335	535	25·3
OM	544	391	602	21·8
CWC	464	375	516	19·6
Non-ammonia-N	−2·7	−6·6	−7·4	1·00
Microbial synthesis†				
g N/d	21·9	20·8	29·8	1·54
g N/kg OM apparent loss in stomach	40·3	53·2	49·4	1·70
Food N escaping rumen digestion‡				
g/d	8·0	13·0	23·5	1·53
g/g N intake	0·28	0·46	0·50	0·058
Turnover time of ^{103}Ru (h)	18·6	12·1	10·4	1·03
Dilution rate of ^{51}Cr (/h)	0·0684	0·115	0·136	0·0054

* For details, see Table 1.
† Estimated by reference to organic ^{35}S.
‡ Estimated as flow of non-ammonia-N from stomach less microbial N and gastric secretions (Weston & Hogan, 1967).

Digestion in the stomach and intestines

The turnover time of ^{103}Ru-P and the dilution rate of ^{51}Cr-EDTA in the stomach (reticulo-rumen, omasum and abomasum) were reduced ($P < 0·01$) and increased ($P < 0·001$) respectively for the CL and CH treatments when compared with the WL treatment (Table 2). There was a further increase ($P < 0·05$) in the dilution rate of ^{51}Cr-EDTA when cold-exposed sheep were given more food (CH *v* CL treatments). By contrast, the retention time of ^{103}Ru-P in the intestines was significantly reduced ($P < 0·001$) in sheep given the higher level of intake (CH treatment) and was not significantly influenced by cold exposure (WL *v* CL treatments) (Table 3).

The flow through the abomasum of DM, OM, CWC and non-ammonia-N was significantly greater ($P < 0·05$), and digestion in the stomach of these constituents was correspondingly reduced for the CL treatment when compared with the WL treatment (Table 2). If the results are expressed relative to intake in order to compare digestion in treatments with differing intake, it is found that an additional 0·13–0·14 of DM intake, 0·12–0·14 of OM intake, 0·10–0·17 of CWC intake, and 0·06–0·14 of non-ammonia-N intake escaped digestion in the stomach of sheep in the CL and CH treatments when compared to warm sheep. The amounts of OM and non-ammonia-N digested in the intestines in the CL treatment were higher ($P < 0·05$) than in the WL treatment, but there were no significant differences in the amounts of DM and CWC digested (Table 3). More ($P < 0·05$) DM, OM, CWC and non-ammonia-N

Table 3. *Digestibility of dry matter (DM), organic matter (OM), cell wall constituents (CWC), and non-ammonia-N (NAN), and retention time of ^{103}Ru in the intestines of sheep given brome grass (Bromus inermis) pellets*

(Mean values with their standard errors for six sheep/treatment)

	Treatments*			
	WL	CL	CH	SE of mean
^{103}Ru retention time in intestines (h)	17·4	18·2	12·2	0·53
Intestinal digestion:				
DM digestibility				
g/d	295	401	601	46·6
g/g DM leaving stomach	0·329	0·369	0·330	0·0127
OM digestibility				
g/d	218	303	466	21·3
g/g OM leaving stomach	0·286	0·325	0·295	0·0135
CWC digestibility				
g/d	42	66	141	16·7
g/g CWC leaving stomach	0·095	0·124	0·142	0·0113
NAN digestibility				
g/d	20·1	23·6	34·9	1·02
g/g NAN leaving stomach	0·651	0·677	0·642	0·0113

* For details, see Table 1.

were apparently digested in the intestines in the CH treatment than in either the CL or WL treatments. The increased digestion in the intestines of the sheep in the CL treatment compared with the WL treatment compensated for 0·59, 0·55 and 0·27 respectively of the reduction of the amount of DM, OM and CWC digested in the stomach. Expressed relative to the amounts of DM, OM, CWC and non-ammonia-N flowing through the abomasum, there were no significant treatment differences in apparent digestibility in the intestines of any constituent, with the exception that the intestinal digestibility of CWC in the CH treatment was higher ($P < 0·05$) than in the WL treatment.

Production of microbial N, methane and VFA in the rumen

The quantity of N incorporated into microbes, estimated by reference to the flow of organic ^{35}S through the abomasum, did not differ significantly between WL and CL treatments, but was significantly greater ($P < 0·01$) in the CH than in the other treatments (Table 2). The efficiency of microbial N production (g/kg OM apparently lost in the rumen) was greater ($P < 0·01$) for cold-exposed sheep than for warm sheep.

The rates of production in the rumen of VFA and methane in the CL treatment were significantly ($P < 0·05$) lower than in both WL and CH treatments, but there were no significant differences between WL and CH treatments (Table 4). Rumen methane production (M, mol/d) was related to VFA production (V, mol/d) according to the equation:

$$M = 0·250V - 0·072 \quad (r\ 0·83, \text{ residual SD } 0·18).$$

Production of methane in the rumen represented 0·81, 0·74, and 0·68 of total methane production for the WL, CL and CH treatments respectively (Table 4), with the differences between all means achieving significance ($P < 0·05$). VFA production and methane production in the rumen were related to the amount of OM apparently digested in the rumen

Table 4. *Saliva production, water volume and turnover, volatile fatty acid (VFA) and methane production, and VFA concentration and proportion of individual VFA in rumen of sheep given brome grass (Bromus inermis) pellets*

(Mean values with their standard errors for six sheep/treatment)

	Treatments*			
	WL	CL	CH	SE of mean
Saliva volume (l/d)	6·7	5·2	6·9	1·2
Water turnover in rumen (l/d)	12·06	12·61	19·23	0·110
Rumen volume (l)	6·93	4·40	5·79	0·287
VFA production (mol/d)	7·46	5·97	7·72	0·348
VFA concentration (mmol/l)	94·7	99·6	96·9	5·21
Proportions of individual VFA (mmol/mol):				
Acetic	668	658	637	4·8
Propionic	168	212	218	6·8
Isobutyric	8·5	7·2	6·3	0·32
Butyric	137	105	123	4·7
Isovaleric	8·0	5·8	4·6	0·48
Valeric	11·3	12·0	12·2	0·50
Rumen methane production (mol/d)	1·86	1·29	1·94	0·082
Post-rumen methane production (mol/d)	0·44	0·48	0·92	0·065
Rumen methane, mol/mol of total methane	0·81	0·74	0·68	0·017

* For details, see Table 1.

(D, g/d) according to the equations:

$$V = 0{\cdot}00809\,D + 2{\cdot}903 \quad (r\ 0{\cdot}77, \text{ residual SD } 0{\cdot}70)$$

and

$$M = 0{\cdot}00277\,D + 0{\cdot}273 \quad (r\ 0{\cdot}89, \text{ residual SD } 0{\cdot}15).$$

Total VFA concentrations did not differ significantly between treatments, but rumen volume in the CL treatment was lower ($P < 0{\cdot}01$) than in the CH treatment which in turn was less ($P < 0{\cdot}05$) than in the WL treatment (Table 4). The molar proportions of individual VFA varied between treatments as follows: acetic acid, WL > CH ($P < 0{\cdot}05$); propionic acid, CL > WL, CH > WL ($P < 0{\cdot}05$); butyric acid, WL > CL ($P < 0{\cdot}05$); and isovaleric acid, WL > CL, WL > CH ($P < 0{\cdot}05$).

The estimated volume of saliva secreted, 5–7 l/d, did not differ significantly between treatments, but there was a higher turnover ($P < 0{\cdot}001$) of water in the rumen of sheep given the CH treatment. Allowing for water entering the rumen in food, saliva and drinking water, entry of approximately 1·9, 6·4 and 5·4 l/d remained unaccounted for in the WL, CL and CH treatments respectively.

Digestion and transformations of N

The concentration of rumen ammonia was greater ($P < 0{\cdot}05$) in warm sheep than in cold-exposed sheep, but there were no significant differences between treatments in the concentration of plasma urea (Table 5).

Exposure to cold increased ($P < 0{\cdot}05$) the amount of non-ammonia-N apparently gained from endogenous sources in the stomach by 3·9–4·7 g/d (Table 2). Allowing for

Table 5. *Concentration and rates of irreversible loss of rumen ammonia and plasma urea nitrogen, total entry rate and recycling of rumen ammonia, transfer of plasma urea-N to rumen ammonia, and microbial N production measured by reference to ^{15}N in sheep given brome grass (Bromus inermis) pellets*

(Mean values with their standard errors for three sheep/treatment except where indicated)

	Treatments*			
	WL	CL	CH	SE of mean
Concentration of rumen ammonia (mg N/l)‡	100	78	82	4·4
Concentration of plasma urea (mg N/l)‡	152	145	156	12·2
Continuous infusion:				
Irreversible loss of rumen ammonia (g N/d)	19·0	17·9	26·9	0·56
Irreversible loss of plasma urea (g N/d)	24·0	21·1	35·9	0·79
Proportion of plasma urea-N derived from rumen ammonia	0·479	0·404	0·389	0·0210
Proportion of rumen ammonia-N derived from plasma urea	0·327	0·440	0·309	0·0074
Transfer of plasma urea-N to rumen ammonia (g/d)	7·32	9·55	9·42	0·286
Single injection:				
Irreversible loss of rumen ammonia (g N/d)	18·1	16·5	27·1	0·71
Total entry rate of rumen ammonia (g N/d)	29·8	27·1	41·2	1·23
Proportion of bacterial N derived from ammonia	0·50	0·58	0·63	0·032
Microbial N production (g/d)	19·5	18·6	28·1	1·08
Microbial N/kg OM apparent loss in stomach	35·9	50·9	45·2	2·67
Irreversible loss of rumen ammonia (g/g N intake)‡	0·66	0·61	0·57	0·024
Rumen recycling of ammonia†:				
g N/d	10·6	8·9	13·0	—
g N/g microbial N produced	0·54	0·48	0·46	—

* For details, see Table 1.

† Calculated as total entry rate – irreversible loss rate – recycling of N to the ammonia pool via plasma urea.

‡ Mean of six sheep/treatment.

abomasal secretions (1 g N/d, Weston & Hogan, 1967), and using the value for microbial N production derived using ^{35}S as a marker, it may be calculated that the amount of food N escaping fermentation in the rumen was 0·28 g N/g N intake for warm sheep, compared to 0·46–0·50 g N/g N intake for cold sheep (Table 2). This difference was significant ($P < 0·05$). Production of ammonia (g N/g N intake) in the rumen was greater ($P < 0·05$) in the WL treatment than in the CH treatment (Table 5).

The irreversible loss rate of rumen ammonia was 0·61–0·66 of the total entry rate of rumen ammonia for all treatments. After allowing for ^{15}N recycled to the ammonia pool by way of plasma urea, the intraruminal recycling rate of ammonia-N appeared to be greater relative to microbial N production in warm sheep than in cold sheep (Table 5). In addition, a

Table 6. *Comparison of molar growth yield of rumen microbes/mol ATP (Y_{ATP}) observed in sheep given brome grass (Bromus inermis) pellets with Y_{ATP} values predicted as discussed on p. 114. Results using ^{35}S or ^{15}N as a microbial marker and ^{51}Cr-EDTA or ^{103}Ru-P dilution rates as an approximation of microbial specific growth rate are compared*

	Treatments*		
	WL	CL	CH
^{35}S-based results			
Observed Y_{ATP}	16·2	19·3	21·4
(1) ^{51}Cr-EDTA dilution rate:			
Maintenance coefficient†	0·00149		
Predicted Y_{ATP}		18·9	19·6
(2) ^{103}Ru-P dilution rate:			
Maintenance coefficient†	0·00117		
Predicted Y_{ATP}		18·5	19·2
^{15}N-based results			
Observed Y_{ATP}	14·2	17·4	19·5
(1) ^{51}Cr-EDTA dilution rate:			
Maintenance coefficient†	0·00208		
Predicted Y_{ATP}		17·2	18·1
(2) ^{103}Ru-P dilution rate:			
Maintenance coefficient†	0·00164		
Predicted Y_{ATP}		16·7	17·5
Average $\frac{\text{predicted}}{\text{observed}}$ Y_{ATP}		0·97	0·91

* For details, see Table 1.
† g moles ATP/g DM microbes per h.

greater proportion of bacterial N was derived from ammonia in cold-exposed sheep, but the difference was not statistically significant. Microbial N production, determined by reference to ^{15}N flowing from the stomach showed differences between treatments similar to those estimated using ^{35}S as a microbial marker, but ^{15}N-based values were lower by 0·10 ± 0·05 (mean ± SEM) in sheep where both markers were employed.

The transfer of 9·4–9·6 g N/d from plasma urea to the rumen ammonia pool in cold-exposed sheep was significantly greater ($P < 0·05$) than the value of 7·3 g N/d in warm sheep (Table 5).

The rate of irreversible loss of plasma urea was greater ($P < 0·01$) in the CH treatment than in the WL or CL treatments, which did not differ significantly. However, when related to N intake, irreversible loss of plasma urea-N at 0·75–0·77 g N/g N intake was similar in both CH and CL treatments but was 0·85 g N/g N intake in warm sheep. By subtraction of the amount of plasma urea derived from rumen ammonia, it was estimated that the irreversible loss of plasma urea not derived from ammonia was 12·5, 12·6 and 21·9 g N/d for the WL, CL and CH treatments respectively, representing 0·44–0·47 of N intake and 0·72–0·80 of non-ammonia-N apparently absorbed from the gut.

DISCUSSION

Digestibility and site of digestion

The reduction of apparent digestibility of DM and OM in the stomach and gastrointestinal tract of sheep exposed to cold followed the pattern reported previously (Kennedy *et al.* 1976). In addition, a further depression of digestibility was associated with the increased food intake of cold sheep given the CH treatment, but more OM was apparently digested in the gastrointestinal tract in the CH treatment than for sheep maintained at 22–25°.

Direct measurement of the turnover and retention time of the particulate marker, ^{103}Ru-P, in the stomach and in the intestines has confirmed previous suggestions (Kennedy *et al.* 1976; Westra & Christopherson, 1976) that the increased rate of passage in cold-acclimated sheep is attributable to increased rate of passage of stomach digesta and increased reticulo-rumen motility. Partition of methane production between the rumen and postruminal tract as an index of fermentative activity also indicates that, for sheep at constant intake, rumen methane production was decreased by 0·3 as a result of cold exposure, but postruminal methane production did not change significantly. For sheep on the CH treatment, increased postruminal methane production occurred simultaneously with increased digestion of OM and CWC in the intestines. If the ratio of methane production to the amount of OM apparently digested in the rumen for each treatment was applicable to the postruminal tract, the quantity of postruminal methane produced in the WL, CL and CH treatments respectively would indicate apparent digestion of 128, 145 and 285 g OM, which represents 0·48–0·61 of the OM measured to have been apparently digested in the intestines. The remainder, 0·39–0·52, of the amount of OM apparently digested in the intestines was similar to the proportion, 0·47–0·58, attributed to apparent absorption of protein (non-ammonia-N × 6·25) from the intestines. The 0·19 of methane production arising from the postruminal tract of warm sheep in this study was higher than the value of 0·13 obtained by Murray *et al.* (1976) for sheep given 792 g lucerne (*Medicago sativa*) chaff/d. This difference probably reflects the relative availability of substrates for microbial fermentation in the intestines of the sheep due to increased passage of material from the stomach of our sheep given brome grass, caused by both the higher intake and the fact that the brome grass had been ground. The finding that postruminal methane may be 0·3 of total methane produced emphasizes the importance of microbial fermentation in the caecum and colon in over-all digestion of the brome grass diet. The results from the present experiment suggest that the isotope dilution technique of Murray *et al.* (1976) is a valuable tool for the study of sites of OM digestion in ruminants, and could be extended to monitoring changes in digestion caused during adaptation of ruminants to cold stress.

Rumen digestion and microbial synthesis

The efficiency of microbial N synthesis in the rumen measured by reference to both ^{35}S and ^{15}N, was significantly increased in cold-exposed sheep, as reported earlier (Kennedy *et al.* 1976). Measurement of VFA production in the present experiment facilitates estimates of molar growth yields of rumen microbes/mol available ATP (Y_{ATP}), assuming that ATP yield/mol VFA produced approximated 2·3 (Leng, 1974), and given the mean value, 0·08 g N/g DM in microbial material isolated from abomasal digesta in this experiment. Values of Y_{ATP} thus calculated for WL, CL and CH treatments were 16·2, 19·3 and 21·4 respectively when ^{35}S was used as the microbial marker, and 14·2, 17·4 and 19·5 respectively if ^{15}N was used as a microbial marker. If the concepts of Stouthamer & Bettenhaussen (1973) of the influence of specific growth rate of single species of micro-organisms on Y_{ATP}, as described by their equation:

$$\frac{D}{Y_{ATP}} = \frac{D}{25} + m_e,$$

where D = specific growth rate of the organism/h; m_e = maintenance coefficient, g mol ATP/g DM per h, are applied to the mixed rumen microbial population, calculations of maintenance coefficient and the effect of increased rate of passage on the Y_{ATP}, of rumen microbes may be made for the present results. To effect the calculation the measured values of the dilution rates of ^{51}Cr-EDTA and ^{103}Ru-P in the rumen have been used for D. The maintenance coefficient was then calculated from the above equation using D and the observed values of Y_{ATP} for rumen micro-organisms in warm sheep as determined above, to

predict values of Y_{ATP} for microbes in the rumen of cold-exposed sheep. Results (Table 6) indicate that the average predicted values are 0·91–0·97 of observed values. In view of the assumptions and approximations involved in these calculations, we consider that the observed and calculated Y_{ATP} values agree rather well, and that differences in dilution rate per se may be a primary explanation of the wide range of efficiencies of microbial synthesis that have been observed in the rumen (Thomas, 1973). In making the extrapolation of the concepts of Stouthamer & Bettenhaussen (1973) to describe the mixed microbial population of the rumen, it must be recognized that it is unlikely that the complex interactions of microbial species and dilution rate would be fully described by the simple equation employed in the present discussion. For example, changes in the rate of engulfment of bacteria by protozoa, or changes in bacterial population structure induced by treatments, reflected by differences in fermentation pattern, may be associated with differences in the mean maintenance coefficient of the rumen microbes. Certainly the increased proportions of propionic acid in the total VFA and reduced digestion of CWC in the stomach in cold-exposed sheep in the present study are indicative of a reduced dependence of microbes on fermentation of cellulose and hemicellulose. However, from present knowledge of the energetics of fermentation (Leng, 1974) there is little evidence to suggest that any one fermentation pattern gives a greater yield of ATP for coupled microbial growth provided the molar ratio, VFA: methane produced remains constant, as it did in the present study, where the ratio was 4·0–4·6.

The maintenance coefficients (1–2 mmol ATP/g DM in cells per h) for rumen micro-organisms calculated above are remarkably low in view of predation of bacteria by protozoa, and the energetically costly synthesis of bacterial material from simple molecules including ammonia in the rumen. Maintenance coefficients this low have been found during rapid in vitro growth for pure cultures of only a few bacterial species (Stouthamer & Bettenhaussen, 1973). The question arises whether the maintenance costs of rumen micro-organisms are unexpectedly low, or values of Y_{ATP} for rumen micro-organisms are derived from falsely low estimates of ATP production in the rumen.

Application of the concepts of Stouthamer & Bettenhaussen (1973) implies that the increased efficiency of microbial synthesis in the rumen of cold-exposed sheep should be accompanied by a decrease in degradation or turnover of rumen microbes. There was some evidence that recycling of rumen ammonia within the rumen, as measured using single injections of $[^{15}N]NH_4Cl$ (Table 5), was reduced in cold-exposed sheep from 0·54 to 0·46–0·48 g N/g microbial N leaving the stomach. If this value truly indicates the amount of N which is returned to the ammonia pool after previously being synthesized into microbial N, and is not simply an artifact resulting from mixing of injected ^{15}N with secondary ammonia pools within the rumen, then reduced degradation of bacteria in the rumen of cold-exposed sheep may be indicated. However, recycling of ^{15}N to the ammonia pool after metabolism in the rumen wall (Havassy, Boda, Kosta, Kuchar & Rybosova, 1974), or after release from nitrogenous compounds in protozoa derived from bacteria would be included in the estimate of intraruminal recycling of ammonia-N.

Nitrogen transformations

The measurement in the present experiment of the large transfer of N from plasma urea to the rumen ammonia pool (7·3–9·6 g N/d) of sheep given brome grass pellets is in contrast to substantially lower values (1·2–1·3 g N/d) measured using the same technique for sheep given lucerne hay (Nolan & Leng, 1972; Nolan, Norton & Leng, 1976). Nolan & Leng (1972) suggested that salivary transport could account for the majority of the transfer in their studies. It is evident from the saliva output measured in the present experiment that salivary transfer could account for only a small proportion of urea-N transfer into the rumen. Differences in concentration of rumen ammonia and blood flow to absorptive sites in the

rumen wall in sheep given different diets in the two studies may account for the differing estimates of urea transfer. Certainly the enhancement of urea transfer, evident when sheep were exposed to cold in the present experiments, was associated with a decrease in rumen ammonia concentration, and with an increased net influx of body water into the rumen.

The greater proportion of bacterial N derived from ammonia in cold-exposed sheep than in warm sheep is in agreement with the results of Mathison & Milligan (1971), who found that there was an inverse relationship between rumen ammonia concentration and the proportion of bacterial N derived from ammonia. This finding was attributed to an increased availability of amino acids for direct incorporation into rumen bacteria under conditions which also promote an increased concentration of rumen ammonia. However, the bacteria isolated from only the fluid portion of rumen contents may not be representative of the total population, and thus this conclusion should not be extrapolated to all rumen bacteria.

The lower estimate of microbial N production obtained using ^{15}N instead of ^{35}S as a microbial marker may be a result of secretion in the omasum or abomasum of organic ^{35}S from endogenous sources, or passage of organic ^{35}S in bacterial exudates from the rumen. We have previously noted that there is a large discrepancy in microbial N estimates using the two markers when sulphate is infused into the rumen (Kennedy & Milligan, 1978). Use of [^{35}S]methionine (Beever, Harrison, Thomson, Cammell & Osbourn, 1974) as a bacterial marker may circumvent this problem.

The mechanism by which cold exposure influences the rate of passage of digesta through the stomach of sheep appears to involve the thyroid gland (Westra & Christopherson, 1976) and further studies on this aspect are in progress in this laboratory. The adaptive significance of the increased rate of passage in cold-exposed sheep is revealed when calculations are made of OM digestion in the gut of warm and cold-exposed sheep. The retention time of ^{103}Ru-P in the gut (retention time in the rumen plus retention time in the intestines) was decreased by 0·16 in the present experiment, from 36·0 to 30·3 h, in sheep exposed to cold at a constant level of intake. If this decrease is accompanied by a proportional increase in food intake of sheep allowed to feed *ad lib.* when exposed to cold (Christopherson, 1976), it may be calculated that the quantity of OM that would apparently be digested in the gut would increase by 0·04. For this calculation it was assumed that the direct effect of cold would reduce OM digestibility from 0·581 by 0·053 units, and that the increased intake would lead to a further reduction of 0·008 units, assuming linearity between food intake and OM digestibility at the intake levels used in the present experiment. It is likely that an increase in metabolizable energy intake would be even more marked in cold-exposed sheep, since methane production in warm sheep was greater than in cold-exposed sheep when expressed relative to OM digested in the whole tract. In addition, more nutrients would, as a result of escape from rumen fermentation, be made available to the tissues of the host directly from dietary material in cold-exposed sheep.

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