Studies of the large intestine of sheep

1. Fermentation and absorption in sections of the large intestine

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1. Fermentation and absorption of constituents of digesta in segments of the large intestine of sheep given different diets were studied by analysis of gut contents obtained at slaughter after a period during which the sheep had been administered a non-absorbable gut marker.

2. In sheep given chopped, dried lucerne (*Medicago sativa*) there was net absorption of water throughout the large intestine with concomitant increases in the proportion of dry matter (DM) and organic matter (OM). There was net disappearance of 62 g OM, 1·66 g non-urea non-ammonia-nitrogen (NU–NAN) and 0·6 g (urea + NH_3)-N in the caecum and proximal colon. There was no significant change in OM and NU–NAN flow through the remainder of the large intestine but there was a net disappearance of 0·3 g NH_3–N. There was also net appearance of volatile fatty acids (VFA) in the caecum, most of which was apparently absorbed before the rectum.

3. Metabolism in the caecum was also studied in sheep grazing fresh pasture or consuming one of three sugar cane–bagasse-based diets, or barley pellets. In the lucerne- and pasture-fed sheep there was a net disappearance of approximately 0·5 g NH_3-N/d from the caecum, while in sheep fed on bagasse plus urea, 1·4 g NH_3-N/d was apparently absorbed from this region. The addition of fish meal to this latter diet resulted in apparent disappearance of 5·3 g NH_3-N/d from the caecum and proximal colon.

4. There was apparent loss of NU–NAN from the caecum of sheep on all diets except the barley diet. With the latter diet there was a net gain of 1 g NU–NAN/d which was associated with relatively high VFA concentration and production; taken together these results indicate that microbial fermentation in the caecum was more extensive in the sheep fed on the barley diet than in those fed on the other diets.

5. The proportions of individual VFA in digesta from the rumen and caecum of lucerne-fed and pasture-fed sheep and in digesta from the caecum of sheep given the bagasse-based or barley diets are also reported and discussed.

6. In general the results indicate that the caecum and to a lesser extent the proximal colon were the major regions of fermentation and absorption of the components of the digesta in the large intestine.

Although there is considerable information obtained from sheep prepared with cannulas in the ileum on the net disappearance of materials between the ileum and the rectum, the only information concerning the fermentation and absorption from different regions of the large intestine is that obtained by Elsden *et al.* (1946) and Williams (1965). Interpretation of both these studies is limited because concentrations rather than apparent appearance and disappearance of digesta components were measured. However, it has generally been assumed from the anatomical structure, the relative retention times of digesta (Grovum & Hecker, 1973) and the ratio, volatile fatty acid (VFA) concentration: dry matter (DM), that most fermentative and absorptive activity occurs in the caecum and proximal colon rather than in the more distal regions of the large intestine.

This study was therefore undertaken to obtain more information on fermentation of organic matter (OM) and nitrogenous components and absorption of ammonia and VFA in various regions of the large intestine of sheep consuming various diets. Sheep consuming lucerne (*Medicago sativa*) hay, sugar cane–bagasse-based diet (with or without addition of supplements), barley, or a grass–legume pasture were studied.

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EXPERIMENTAL

General

In each of three experiments an indigestible, non-absorbable marker was administered to sheep for sufficient time for its equilibration throughout the gut contents. Sheep were then anaesthetized, the abdomen was opened and the gut tied at selected points as quickly as possible. Samples of digesta were immediately obtained from each tied-off gut segment. The quantity and concentration of digesta components in these segments was obtained from analysis of the digesta, and the flow-rates of these components through each segment were calculated by reference to the non-absorbable marker.

Experiments

Expt 1. Six mature (2–3 years) Merino wethers of similar genetic origin and live weight (29–35 kg) were each prepared with a rumen cannula (Hecker, 1969) and a caecal cannula (66 mm × 17 mm). The latter was implanted approximately one-third of the distance from the ileo-caecal junction to the caecal pole, and exteriorized through the lower right flank (MacRae *et al.* 1973). An infusion catheter (Silastic; Dow Corning, Michigan, USA; internal diameter 1·02 mm) into the caecum was securely attached to the caecal wall midway between the cannula and the pole of the caecum. A post-operative recovery period of at least 8 weeks was allowed. Catheters were inserted into one or both jugular veins 12–18 h before experiments were commenced.

The sheep were housed indoors in metabolism crates under continuous lighting, and an anthelmintic (Thibenzole; Merck Sharpe & Dhome, Sydney, Australia) was administered regularly. Chopped sun-cured lucerne hay (800 g/d, air-dry) was fed for at least 60 d before all experiments and was fed in equal hourly portions for at least 7 d before and also during all experiments. The lucerne hay contained 905 g DM/kg air-dry material and 918 g OM and 27·3 g N/kg DM.

^{51}Cr complexed with ethylenediaminetetra acetic-acid (^{51}Cr-EDTA) was continuously infused into the rumen (20 μCi plus carrier Cr-EDTA in 1·1–1·2 l water/d) for 3 d after which the animals were slaughtered to enable the contents of the whole digestive tract to be sampled. Continuous infusions of ^{14}C and ^{15}N tracers (to provide information to be reported later) were made into the caecum in 1·1–1·2 l water/d for 18 h before slaughter in five sheep, and into the jugular vein in 0·08–0·12 l saline (0·15 M-sodium chloride)/d in the sixth sheep.

On the day of slaughter blood and rumen samples were taken at 09.00, 10.00 and 11.00 hours. Blood samples (10–20 ml) were placed into heparinized tubes which were immediately centrifuged (3000 ***g*** for 15 min) and the plasma stored at −20°. Rumen fluid samples (10 ml) free of large particulate matter were taken by suction through a nylon gauze-covered perforated tube in the ventral sac of the rumen. Samples were acidified (0·2 ml, 18 M-sulphuric acid) before storage at −20°.

Immediately after the last blood sample was obtained the sheep were anaesthetized by intravenous administration of sodium thiopentone (Intraval; May and Baker, Dagenham, UK; 50 g/l; 0·7 ml/kg live weight). The abdomen was opened and the gut was ligated with tape at selected sites as quickly as possible.

The gut was divided into the following segments: (1) the rumen was isolated by placing ties at the distal end of the oesophagus and around the reticulum-omasal junction; (2) the small intestine was tied immediately proximal to the ileo-caecal junction and approximately 2–3 m proximal to this point; the intervening intestine was termed the ileum segment; (3) a tie was placed around the caecum at the ileo-caecal junction; the intestine between this tie and the pole of the caecum was termed the caecum segment; (4) the proximal colon was

divided into two approximately equal lengths; the proximal segment was designated PCa and the distal segment PCb; (5) the centripetal turns of the spiral colon were divided into two approximately equal lengths; the proximal segment was designated SCa and the distal segment SCb; (6) the centrifugal turns of the spiral colon, the descending colon and the rectum were divided into four approximately equal lengths, and the most distal segment divided again into two approximately equal lengths. From the proximal end these segments were designated SCc, SCd, SCe, SCf and Rect.

The digesta in each segment were gently stripped by hand into tared beakers which were then immediately reweighed.

The digesta were then mixed and subsampled. For ^{51}Cr-EDTA analysis 1–4 g was transferred into a tared gamma counting vial using a glass tube 7 mm internal diameter; for DM approximately 10 g was dried to constant weight at 70°, and for total N approximately 5 g was mixed with 5 ml 0·5 M-sulphuric acid. Another subsample (approximately 10 g) was mixed with 0·92 M-sulphosalicyclic acid (10 ml) and centrifuged at 1000 ***g*** for 1 min to remove large particulate matter. The supernatant solution was recentrifuged (16000 ***g*** for 20 min) and the resulting supernatant solution stored at −20° for subsequent ammonia-N, urea-N and VFA analysis.

All of these procedures were normally completed within 30 min of anaesthetization.

Expt 2. Six half-sib Merino sheep grazing a phalaris (*Phalaris tuberosa*), white clover (*Trifolium repens*) pasture were used. Anthelmintic (Rametin; Bayer, Botany, Australia) was administered 38 and 31 d before slaughter. Each sheep was drenched with a 1 g capsule of chromic oxide at 08.00 and 18.00 hours for 6–8 d before slaughter.

On the day of slaughter sheep were removed from the pasture at 09.00 hours and sampling commenced. A single jugular blood sample was obtained by venous puncture before administration of anaesthetic as described in Expt 1. Samples of digesta were obtained from the rumen, ileum, caecum, proximal colon and rectum using the procedures described for Expt 1. The pH of these samples was measured with a glass electrode immediately after the digesta were removed from the gut.

Expt 3. This experiment was carried out following a 35 d feeding trial (Kempton, 1979) using thirty Dorset Horn × (Border Leicester × Merino) wether lambs (initially 17·6 kg). Lambs had been randomly allocated to dietary treatments (five lambs/diet), housed indoors in individual pens on slatted flooring, vaccinated against clostridial infections (Tasman Vaccine Laboratory Australia Pty Ltd) and drenched fortnightly with an anthelmintic (Nilzan; ICI, Australia).

Five sheep from each of four dietary treatments in the feeding trial (i.e. twenty sheep) were used. The basal diet (diet B1) consisted of a mixture of (g/kg) 500 sugar, 400 sugar-cane bagasse (Murwillumbah Sugar Mills, NSW, Australia) and 100 chopped lucerne hay to which was added (/kg): 5 g NaCl, 5 g Na_2SO_4, 5 g minerals (Sustra-Vet-R; British Veterinary Products, Australia) and 40 g urea. The basal diet was mixed with either 125 g fish meal (diet B2)/kg or 125 g rice bran (diet B3)/kg. The fish meal (104 g N/kg DM) was supplied by Toyo Maru, Japan C/o Fielder's Stock Feed Manufacturers, Tamworth, Australia, and the rice bran (24 g N/kg DM) was supplied by Coprice Feeds, Griffith, Australia. Diet B4 consisted of ground and pelleted barley supplemented with urea and minerals (Fielder's Stock Feed Manufacturers, Tamworth, Australia), and contained 26·5 g N/kg DM.

Faecal OM flow was estimated using Cr_2O_3 as an indigestible, non-absorbable marker. For 5 d near the end of the feeding trial powdered Cr_2O_3 (0·5 g/d) was mixed with the ration, and for the last 2 d of this 5 d period the majority of the faeces was collected and subsampled from each of the five sheep on each diet.

All lambs were fed *ad lib.* once each day until the last 6 d before slaughter when an amount

equal to the mean *ad lib.* intake during the previous 3 d was offered in four equal portions each 6 h for 3 d, then in eight equal portions each 3 h for 3 d. Two animals were selected at random from each dietary group for sampling of gut contents at slaughter. Sampling of blood and gut contents was performed as described for Expt 2.

Laboratory methods

DM, OM and total N content of food, faeces and digesta were determined by standard procedures (Association of Official Agricultural Chemists, 1975).

NH_3 was obtained by steam distillation of digesta samples made alkaline by the addition of 5 ml saturated sodium tetraborate solution. The resulting NH_3 was collected into 5 ml 0·32 M-boric acid and titrated with 0·05 M-H_2SO_4. Plasma and the protein-free supernatant fraction of digesta were analysed for urea-N using an autoanalyser and the diacetyl monoxime method of Marsh *et al.* (1957). For total N analysis, 0·5–1 g dried food or faeces, or the equivalent weight of wet digesta was subsampled and oxidized. Non-urea, non-NH_3-N (NU-NAN) was calculated by subtracting the NH_3-N and urea-N components from the total N concentration.

Milled subsamples (1 mm screen) of dried digesta and faeces were ashed and digested (Stevenson & DeLangen, 1960) and the Cr^{3+} content determined on an atomic absorption spectrophotometer (Perkin–Elmer, Model 360) using an acetylene–nitrous oxide flame.

Total VFA in digesta supernatant solutions was estimated by steam distillation (Annison, 1954) and individual VFA proportions by gas–liquid chromatography (Erwin *et al.* 1961).

The radioactivity of ^{51}Cr-EDTA in digesta and faeces was determined using a gamma spectrometer (Model 3002; Packard Instrument Co., Illinois, USA). Corrections were made for the effect of sample height on efficiency of counting. Infusion solutions were counted under the same conditions as the samples.

Calculations

The flow of any digesta component (X) in the sheep used in Expt 1 was calculated as follows:

$$\text{Flow (X) (g/d)} = \frac{^{51}\text{Cr-EDTA infused (counts/min per d)}}{^{51}\text{Cr-EDTA in digesta (X) (counts/min per g)}}$$

In Expt 2 the faecal OM flow was similarly calculated from the concentration of Cr_2O_3 in the faeces. In Expt 3 the digestibility of each diet was calculated from the faecal Cr_2O_3 concentration, and the faecal flow on the day each sheep was killed was predicted from the digestibility and the mean intake of the previous 3 d. In Expts 2 and 3 the rates of OM flow through the ileum, the caecum and the proximal colon were calculated by assuming that the apparent digestibility between these sites and the rectum was the same as that measured in sheep given the lucerne diet.

The retention time of digesta in each sampled segment was calculated as described by Hecker & Govum (1971), i.e.

$$\text{Retention time (min)} = \frac{\text{weight of wet contents (g)}}{\text{flow of total digesta (g/min)}},$$

where flow of digesta was obtained from the ^{51}Cr-EDTA concentration in each segment.

Statistical analysis

An analysis of variance (ANOVA) was used to test for significant differences among animals, between diets and among sampling sites within each animal. Sites were compared within diets by comparison with the error term of animals × sites. When animals, diets and sampling sites were considered simultaneously in Expt 3, diets were tested against the error

term of the diets × animals interaction. Sites and diets × sites interaction were tested against the error term of animals × diets × sites interaction.

The 5% Studentized Range (Snedecor & Cochran, 1967) was used to separate means into their respective classes when a significant F test was obtained in the ANOVA. A log transformation of the results was used for the ANOVA to reduce heterogeneity of variance.

RESULTS

Variability among animals

The variability of ammonia and VFA concentrations in rumen and caecal digesta was considerable (coefficients of variation 20–31%).

Expt 1. Sheep given lucerne chaff

The mean concentrations and flows of DM, OM, water, NH_3, NU–NAN and VFA at each of eleven sampling sites in the ileum and large intestine are given in Table 1. Progressing distally along the large intestine the pattern of increasing DM content associated with decreasing water flow indicated that approximately 3·0 l water/d, were absorbed from the caecal segment when the 1·1–1·2 l water/d which was infused directly into the caecum of the five sheep receiving tracers into the caecum was included. In the remaining sheep with no infusion into the caecum, the DM content of large intestinal digesta tended to be greater, but the remaining measurements were similar to those for the caecally-infused sheep.

The decrease in flow of 1·6 g NU–NAN plus 0·6 g (urea + NH_3)-N/d between the ileum and PCb segments (Table 1) indicated a net absorption of 2·2 g N/d in the caecum and proximal colon. A further 0·5 g N/d was apparently absorbed between the proximal colon and the rectum. NH_3 concentration tended to decrease (not significantly) from 240 mg N/l in the caecum to 150 mg N/l in the SCa and SCd segments of the spiral colon, and then to increase significantly in the SCf and Rect. segments. However, the NH_3 flow decreased distally from 0·98 g N/d entering the caecum to 0·14 g N/d passing through the rectum.

The difference in OM flow between the ileum and the PCb segments indicated that 62 g OM/d was apparently digested in the caecum and proximal colon, but there was negligible net disappearance in the spiral and descending colon. The pattern of total VFA concentration and flow was similar to that of NH_3, with no change in concentration but a progressive decrease in flow in the more distal segments of the colon indicating that at least 0·1 M VFA/d was absorbed. The percentage of acetate in total VFA was significantly higher ($P < 0·05$) in the rectal fluid (79%) than in the caecal fluid (75%), and there was a concomitant decrease in the proportion of propionate.

The relative proportion of acetate tended to be greater in caecal fluid than in rumen fluid, the proportion of butyrate less and the proportions of propionate and total long-chain acids similar (Table 2).

The retention times of digesta within segments of the large intestine are given in Table 1; the total retention time in the caecum and proximal colon (272 min) was similar to that in the SCe, SCf and Rect. segments (228 min) but much longer than in the SCa, SCb, SCc and SCd segments (118 min).

Expt 2. Sheep given pasture

Although the sheep at pasture were smaller (17·6 ± 0·7 kg) than the wethers fed on lucerne (30·8 ± 1·4 kg), the weights of total wet digesta in the caecum and proximal colon were similar when expressed on a metabolic body-weight ($W_{0·75}$) basis (46 ± 7 and 37 ± 3 g/kg $W^{0·75}$ respectively).

In sheep consuming pasture the flow of (urea + NH_3)–N decreased ($P < 0·05$) from 1·2 g N/d in the ileum to 0·5 g N/d in the caecum and proximal colon, although the

Table 1. *A comparison of the concentrations and flows of various components of digesta obtained from segments of the ileum and large intestine of six sheep fed on lucerne* (Medicago sativa) *and the length of each gut segment, the weight of digesta, and the retention time of digesta in each segment*

	Sampling site												Statistical significance between sites
	Ileum	Caecum	PCa	PCb	SCa	SCb	SCc	SCd	SCe	SCf	Rectum	SEM	
Length of each segment (mm)	2680	240	200	230	420	450	790	810	750	420	460	—	—
Digesta (g)	170	253	126	105	32	23	39	35	49	35	48	—	—
Retention time (min)	—	128	78	66	20	17	37	44	74	60	94	—	—
Water flow (g/d)	4230	2514	2130	2039	1980	1657	1188	870	672	560	455		
	(8·350)	(7·830)	(7·664)	(7·620)	(7·591)	(7·413)	(7·080)	(6·768)	(6·511)	(6·328)	(6·121)	(2·354)	***
Dry matter (g/kg)	84	111	122	130	148	174	215	260	309	353	392		
	(4·431)	(4·710)	(4·804)	(4·871)	(4·996)	(5·157)	(5·369)	(5·561)	(5·734)	(5·866)	(5·972)	(2·330)	***
Organic matter (g/kg DM)	847	863	867	874	886	897	902	902	902	910	916		
	(6·742)	(6·761)	(6·765)	(6·773)	(6·787)	(6·800)	(6·006)	(6·805)	(6·805)	(6·814)	(6·820)	(2·306)	***
Organic matter flow (g/d)	320	256	241	258	292	290	278	265	263	268	263		
	(5·769)	(5·546)	(5·489)	(5·553)	(5·678)	(5·671)	(5·628)	(5·579)	(5·573)	(5·591)	(5·572)	(0·029)	***
NH_3-N concentration (mg N/l)	70†	240	206	199	150	139	145	150	177	260	309		
	(4·243)	(5·480)	(5·329)	(5·291)	(5·008)	(4·931)	(4·978)	(5·013)	(5·177)	(5·562)	(5·733)	(0·101)	***
NH_3-N flow (g N/d)	0·29†	0·60	0·45	0·41	0·30	0·23	0·17	0·13	0·11	0·15	0·14		
	(−1·225)	(−0·505)	(−0·807)	(−0·898)	(−1·216)	(−1·471)	(−1·758)	(−2·035)	(−2·166)	(−1·925)	(−1·963)	(0·109)	***
NU–NAN concentration (g N/kg DM)	17·6	19·5	19·3	18·4	17·0	17·2	17·4	17·1	17·4	17·4	17·5		
	(2·869)	(2·973)	(2·960)	(2·913)	(2·834)	(2·847)	(2·857)	(2·838)	(2·859)	(2·858)	(2·863)	(2·334)	NS
NU–NAN flow (g N/d)	7·09	6·05	5·56	5·47	5·75	6·26	5·91	5·20	5·52	5·53	5·23		
	(1·958)	(1·800)	(1·715)	(1·700)	(1·749)	(1·834)	(1·776)	(1·648)	(1·709)	(1·710)	(1·654)	(0·036)	***
VFA concentration (mmol/l)	6	49	49	47	49	48	46	47	49	48	58		
	(1·790)	(3·890)	(3·891)	(3·840)	(3·898)	(3·879)	(3·836)	(3·845)	(3·982)	(3·880)	(4·062)	(0·060)	***
VFA flow (mmol/d)	27	123	106	98	104	86	59	43	37	30	28		
	(3·291)	(4·812)	(4·665)	(4·587)	(4·649)	(4·454)	(4·085)	(3·772)	(3·599)	(3·394)	(3·344)	(0·083)	***

DM, dry matter; NH_3–N, ammonia-nitrogen; NU–NAN, non-urea, non-ammonia-nitrogen; VFA, volatile fatty acids; NS, not significant.
*** $P < 0·001$.
Analysis of variance was carried out using log transformation of the results. The mean values for each sampling site are the antilogs of the means of the log-transformed results; the values in parentheses are the mean (and SEM) of the log values.
† Ileal concentration of urea plus NH_3 = 230 mg N/l; ileal flow of urea plus NH_3 = 0·98 g N/d.

Table 2. *The concentrations of ammonia and of total volatile fatty acids (VFA) and the proportions of individual VFA in digesta from the rumen and caecum-proximal colon of six sheep fed on chopped lucerne* (Medicago sativa) *or pasture, or in digesta from two sheep fed on bagasse-based or barley diets*

		Concentrations		Proportions of individual VFA (mmol/mol)			
Diet	Site	Ammonia (mg nitrogen/l)	Total VFA (mmol/l)	Acetate	Propionate	Butyrate	Total of other long chain acids
Lucerne	Rumen	156	85	680	190	81	44
	c/c	219	48	740	180	40	44
Pasture	Rumen	232	83	620	210	85	85
	c/c	250	60	730	190	38	45
Basal bagasse	c/c	244	114	740	190	48	20
Bagasse + fishmeal	c/c	463	104	760	170	44	30
Bagasse + rice bran	c/c	392	88	720	190	58	36
Barley	c/c	214	162	620	86	270	27

c/c, Mean of the three sampled sites in the caecum and proximal colon.

concentrations were similar at the two sampling sites (Table 3). There was also an apparent loss of 1·5 g NU–NAN/d between the ileum and the caecum and proximal colon, indicating that there was a net absorption of 2·2 g N/d in the caecum and proximal colon. There was an increase in total VFA concentration and flow between the ileum and the caecum and proximal colon (Table 3), while the relative proportions of individual VFA in both rumen and caecal digesta were similar to those in sheep given lucerne (Table 2).

Expt 3. Sheep given bagasse-based and barley diets

There was a significant difference ($P < 0{\cdot}01$) in faecal OM flow between diets reflecting the different intakes and digestibilities of each diet (Table 3).

The mean concentrations and rates of flow of ileal NH_3 appeared considerably greater in the sheep fed on the barley diet (B4) (223 mg N/l) than in those fed on the bagasse-based diets (B1, B2, B3) (75–89 mg N/l); however, there were no differences in the concentrations and rates of flow of ileal (urea + NH_3)–N (Table 3). The higher NH_3 concentration in the ileal digesta of sheep fed on the barley diet was associated with a lower ileal pH (6·8) than in the sheep fed on the bagasse-based diets (pH 7·9) (Table 3). The concentration of NH_3 in the caecal and proximal colon digesta appeared greater when the bagasse diet was supplemented with fish meal (B2) or rice bran (B3) (463 and 392 mg N/l) than when the basal bagasse diet (B1) or the barley pellets (B4) were given (244 and 214 mg N/l). Also the rate of flow of NH_3 (expressed as g/d or as g/kg faecal OM) tended to be greater with the bagasse-based diets containing fish meal or rice bran (B2 and B3) (Table 3). Although the concentration of (urea + NH_3)–N appeared to be greater in the ileal digesta of the sheep fed on the diets containing bagasse or the barley diet than in sheep fed on lucerne or pasture, the flows of ileal (urea + NH_3)–N appeared to be similar for all diets except the fish meal-supplemented diet which tended to be greater (Table 3).

The concentration of NU–NAN and flows of NU–NAN per unit faecal OM were similar in the caecum and proximal colon of sheep consuming the bagasse-based diets (24–25·3 g N/kg DM; 23–31 g N/kg faecal OM), but were lower as a group than these measurements for the barley diet (38 g N/kg DM; 45 g N/kg faecal OM) (Table 3). The rate of flow of NU–NAN through the caecum and proximal colon was significantly greater (15·4 g N/d)

Table 3. *The concentrations and flows of various components in digesta in the ileum and the caecum and proximal colon (c-pc) of lambs (live weight 17·6 ± SE 0·7 kg) fed on phalaris-white clover pasture (Expt 2) and sheep (25·9 kg ± SE 1·3) fed on sugar cane–bagasse-based diets (B1, B2, B3)† or barley (B4)† (Expt. 3). Mean (± SE) faecal organic matter (OM) excretion rates were 188 ± 18 and 241 ± 36 for Expts 2 and 3 respectively*

(The number of observations at each sampling site is given in parentheses)

		Ammonia + urea			NU–NAN			VFA			
		Concentration (mg N/l)	Flow (g N/d)	Flow (g N/kg faecal OM)	Concentration (g N/kg DM)	Flow (g N/d)	Flow (g N/kg faecal OM)	Concentration (mmol/l)	Flow (mmol/d)	Flow (mmol/kg faecal OM)	pH
Expt 2											
Pasture	Ileum (6)	297	1·2	6·2	19	5·7	31	10	34	180	7·9
	c-pc (18)	250	0·5	2·5	20	4·2	23	60	112	590	7·2
	Statistical significance of difference between sites	*	***	***	NS	*	*	***	**	***	***
Expt 3											
B1	Ileum (2)	415	1·4	7·9	21	5·3	31	5	17	100	7·9
B2	Ileum (2)	416	1·9	6·9	30	12·6	48	5	25	90	7·9
B3	Ileum (2)	338	1·2	6·7	21	5·9	32	7	26	140	7·9
B4	Ileum (2)	362	1·2	3·5	29	14·4	44	42	128	370	6·8
B1	c-pc (6)	244	0·3	1·8	25	5·0	28	114	160	880	6·5
B2	c-pc (6)	463	1·1	4·0	25	8·2	31	104	238	900	6·8
B3	c-pc (6)	392	0·5	2·8	24	4·2	23	88	113	610	6·8
B4	c-pc (6)	214	0·4	1·2	38	15·4	45	162	330	930	6·1
	SEM and statistical significance of difference between sites within Expt 3	43	0·13	0·04	2·5	1·07	3·34	13	32	9	0·1
		NS	*	*	NS	NS	NS	*	*	*	*
SEM and statistical significance of difference between diets within Expt 3		72	0·23	0·07	4·8	2·71	7·64	43	106	24	0·6
		NS	*	*	NS	*	NS	NS	NS	NS	NS

N, nitrogen; OM, organic matter; DM, dry matter; NU–NAN, non-urea, non-ammonia-nitrogen; VFA, volatile fatty acids; NS, not significant.
*$P < 0·05$; ** $P < 0·01$; *** $P < 0·001$

† See text, p. 219.

for the barley diet (B4) than for the other diets, and the flow with the fish meal diet (B2) also tended to be greater than with the basal (B1) or rice bran (B3) diets. The apparent loss of NU–NAN between the ileum and the PCb segments was considerably greater (4·4 g N/d) with the fish meal diet (B2) than with the basal (B1) or rice bran (B3) diets (0·3–1·7 g N/d). In contrast there was an apparent gain of 1·0 g N/d in the caecum and proximal colon of sheep fed on the barley diet.

The total VFA concentration in caecal digesta appeared to be highest and the caecal pH lowest with the barley diet (B4) (Table 2). Since the flow of DM was also high, the estimated flow of total VFA was twice than that for the basal (B1) or rice bran (B3) diets, and considerably greater than that of the fish meal (B2)-supplemented diet. Butyrate comprised 4–6% of the total VFA in sheep on the bagasse-based diets but 27% of the total VFA for the barley diet. Corresponding values for acetate were 72–76% and 62%, and for propionate 17–19% and 9% respectively.

DISCUSSION

Validity of techniques

The technique of calculating apparent appearance or disappearance of digesta components using the concentration of a continuously-administered indigestible marker is well established (Faichney, 1975). Since there is little difference in the retention time of solid and liquid phases in the large intestine (Coombe & Kay, 1965; Grovum & Williams, 1973), the calculation of flow of all digesta components using a single marker is unlikely to have introduced errors. Weller *et al.* (1971) also showed that solid and liquid phases were sampled representatively in the large intestine at slaughter.

Analysis of components in digesta obtained in slaughter experiments may be influenced by shedding of the mucosa before samples are removed from the gut (Badawy *et al.* 1957, 1958). However, the shedding is not initiated during anaesthesia before the death of the animal; it is a process progressing distally from the duodenum, and it does not begin in the caecum and colon until approximately 20 min after death (Fell, 1961). In these experiments gut sections were quickly removed from the anaesthesized animal and emptied into the sample containers, and it is therefore unlikely that there was time for shedding of the mucosa to have occurred. Furthermore, since shedding would occur in the ileum before the caecum (Fell, 1961) and since the ratio, mucosa surface area:digesta mass would be much larger in the ileum, the expected effect of mucosal shedding would be to increase ileal NU–NAN flow more than caecal NU–NAN flow. This would lead to an over-estimation of the apparent digestion of OM and N in the large intestine. However, with the sheep given lucerne in the present study the apparent digestion in the large intestine of 62 g OM and 2·2 g total N/d was somewhat less than that measured in lucerne fed on sheep prepared with ileal cannulas, where there was an apparent digestion of 137 g OM and 5·7 g total N/d (Coelho da Silva *et al.* 1972; Thomson *et al.* 1972).

It is difficult to assess whether ideal steady-state conditions of digesta flow were approached sufficiently closely for a single sample of digesta from any gut segment to provide a precise estimate of the mean daily flow of digesta in that segment. The continuous feeding and lighting conditions used during Expts 1 and 3 should have resulted in a relatively constant flow of digesta through the gut (Minson, 1966). The absence of any appreciable diurnal variation in concentrations of marker or fermentation end-products in vivo in the same sheep that were later used in Expt 1 (R. M. Dixon, unpublished results) suggested that errors from this source were within acceptable limits.

In Expts 2 and 3 the use of faecal OM flow to calculate flow through the ileum and caecum-proximal colon by assuming the same apparent digestibility of OM between the ileum or caecum and the rectum as was measured in the sheep fed on lucerne was a source of error. It was originally intended to calculate flows from marker concentration in digesta,

but large variability among sites suggested that marker or digesta flows or both were not constant in the grazing sheep. Consequently the estimated flows of N and VFA in Expt 2 and to a lesser extent in Expt 3 must be interpreted cautiously.

Variability among animals

The similarity of the variability in the NH_3 and VFA concentrations in the rumen and the large intestine was unexpected since greater variability in the caecum than in the rumen occurred in previous experiments (Williams, 1965; Faichney, 1968, 1969). A similarly high variability in caecal measurements was observed in vivo during other experiments with these sheep (R. M. Dixon, unpublished results) and consequently appears to be characteristic of the large intestine even with sheep in steady-state. Possibly the irregular flow of digesta into and out of the caecum and proximal colon (Hogan & Phillipson, 1960; MacRae *et al.* 1973) is the cause of much of this variation.

Importance of various parts of the large intestine

These experiments support the concept that the primary sites of fermentation in the large intestine are the caecum and the proximal colon. The result is to be expected since this segment contained 65% of all the digesta in the large intestine in the sheep fed on chopped lucerne, and since digesta passing out of the small intestine are first exposed to fermentation in this region.

In sheep given lucerne, the decrease in flows of OM, NU–NAN and (urea + NH_3)–N between the ileum and the caecum and proximal colon, and the concurrent increase in VFA concentration and flow of VFA indicated that considerable fermentation was occurring in this region. The apparent loss of 2·2 g N/d also indicated considerable NU–NAN degradation and absorption of NH_3. This value and other values for absorption of NH_3 or VFA represent net loss, and NH_3 derived from endogenous inputs of urea or mucous secretion or VFA produced from OM in secretions would not be taken into account; the total rates of absorption of NH_3 and VFA are therefore probably somewhat greater than the net values reported.

The flows of OM and NU–NAN and the concentrations of total VFA and NU–NAN did not change significantly throughout the spiral colon or descending colon. If further NH_3 production from, for example, urea hydrolysis or VFA production from fermentation were occurring in this part of the gut, actual absorption would have been greater than that indicated by the apparent decrease in flows of NH_3 and VFA. That some NH_3 production did occur is indicated by the increased NH_3-N concentration in the Scf and Rect. segments. This was associated with an increased digesta retention time in this part of the gut. Since the digesta have already been exposed to fermentation, it is unlikely that appreciable fermentation from substrates other than perhaps endogenous material or microbes would occur.

The retention times in the large intestine of the sheep fed on lucerne were less than those reported by Grovum & Hecker (1973) for sheep consuming a similar quantity of lucern chaff. However, the smaller body-weights of the sheep used in the present experiment (29–35 kg *v.* 40–43 kg) and different digestibilities of the chopped lucerne may have contributed to these differences.

Differences between diets

Although differences in body-weight, genetic origin and food intake of the sheep used in the different experiments confounded the results obtained with the various diets, some comparisons between diets can be made. The expression of flow-rates of digesta components as a function of faecal OM flow (the large intestinal factor most closely correlated with food

intake) was used to take account of the differences between diets in food intake. A comparison of the results for the two diets studied in most detail (lucerne and phalaris-white clover pasture) suggested that most of the differences in the caecal measurements could be explained in terms of the different intakes.

The concentrations of NU–NAN, and of flows of NU–NAN per unit faecal OM for the sheep fed on lucerne or pasture were similar, although there were large differences between the daily rates of flow of NU–NAN. This suggests that the difference between diets was a function of the different amounts of OM flowing through the large intestine. In contrast, the NH_3 and VFA concentrations and rates of flow did not differ greatly between diets except when expressed per unit faecal OM flow. Consequently, it appears that the concentrations and rates of flow of NH_3 and VFA were, for these diets, independent of the different amounts of OM entering the large intestine. This implies that changes in production of VFA or NH_3 do not necessarily result in marked changes in concentration and flow of these substances out of the caecum. This suggestion is consistent with the concept that absorption of NH_3 and VFA from the caecum and proximal colon occurs by concentration dependent mechanisms (Myers *et al.* 1967; Hecker, 1971).

The absorption of total N between the ileum and the proximal colon was 1·4 g N/d with sheep fed on the basal bagasse diet and 5·3 g N/d with the fish meal–bagasse diet. Possibly this was due to a considerable proportion of the fish meal escaping digestion proximal to the ileum (evidenced by the high flow of NU–NAN through the ileum with this diet), and this dietary nitrogenous material being degraded to NH_3 in the large intestine.

The effect of addition of rice bran to the basal bagasse diet was of interest, since responses in live-weight gain in cattle led Leng & Preston (1976) to suggest that starch and lipid in this supplement may have passed through the forestomachs thereby becoming available for absorption from the small intestine. However, if this occurred in the present experiments with sheep there was no evidence that additional substrate reached the large intestine and thereby increased fermentation in this region. It also seems unlikely that the supplement increased the supply of substrate to the small intestine since there was no response to rice bran supplementation in the concurrent lamb growth trial (Kempton, 1979).

It seemed likely that a more extensive large intestinal fermentation might have occurred in the sheep fed on the barley diet as compared to the other forage type diets as a result of starch escaping digestion proximal to the ileum. The concentration of total VFA in caecal digesta increased considerably in sheep fed on barley in both the present study and in that of Ørskov *et al.* (1970). Since pH in the caecum was also relatively low (pH 6·8) the rate of VFA absorption was probably increased (Myers *et al.* 1967), implying that the small increase in VFA concentration may indicate a relatively large increase in VFA production. The net gain of 1·0 g NU–NAN/d between the ileum and the caecum for the sheep fed on barley, compared with a net loss with the other diets, also suggested that bacterial N synthesis was more extensive as a consequence of increased energy availability. In comparison with the high acetate fermentation found in the caecum of sheep fed on the high-forage-type diets, the high butyrate fermentation of the type characteristically observed in the rumen of animals fed high-sugar diets (Leng & Preston, 1976) is of great interest, since in the rumen an increased propionate proportion would be expected to result from fermentation of barley starch. A high butyrate fermentation was also observed by Ørskov *et al.* (1970) in sheep fed on barley, and hence appears to be characteristic of caecal fermentation when starch is available.

Conclusions

This study has shown that the caecum, and to a lesser extent the proximal colon, are the most important regions of fermentation in the large intestine. The spiral and descending

colons were also involved in absorption of water, NH_3 and VFA, but apparent digestion of OM and NU-NAN was negligible.

Considerable variation in fermentation and absorption in the large intestine can result from manipulations with commonly-used diets. Nevertheless the changes in apparent N disappearance and fermentation in the large intestine do not appear to be as extreme as those frequently reported for the rumen, presumably because the extensive modification of dietary materials in the rumen and small intestine would reduce the variability in the amount and type of substrate entering the large intestine.

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